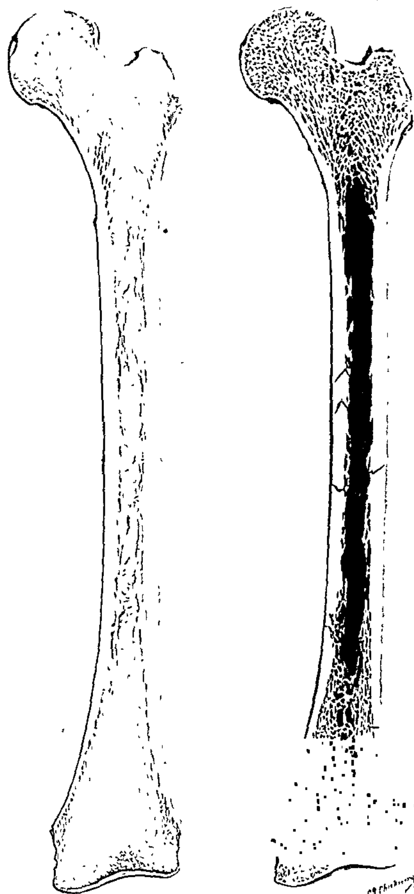


Femoral Marrow Left—Normal Adult Right—Pernicious Anemia.
(From The Army Institute of Pathology, World War I Collection)

An Atlas of
The Blood and Bone Marrow

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285 ILLUSTRATIONS, 42 IN COLOR

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TO

CLAIRE

PREFACE

Over the past two decades hematology has come into its own as a specialty of medicine. The considerable number of textbooks, atlases, and journals devoted exclusively to the blood and blood-forming organs bears witness to this fact. Yet hematology is firmly integrated with virtually every branch of medicine. The general practitioner and specialist alike, to do full justice to their patients, must understand normal and pathological physiology of blood formation and destruction They should have at least a general idea of the multiform manifestations of primary hematopoietic disorders. Still more important, they should know the manner in which the blood-forming organs react to disease elsewhere in the body.

This book was planned in the hope that it may prove useful to clinicians, clinical hematologists, pathologists, and medical technologists. The first section is devoted to the development and normal function of hematopoietic tissues Then, largely through the medium of pictures, I have attempted to correlate changes in the peripheral blood with those in the bone marrow, encompassing most primary diseases of the blood-forming organs, and many other conditions in which the blood and bone marrow are directly affected or react secondarily In the text I have summarized significant clinical features and called attention to adjunct laboratory tests, so that unwarranted reliance will not be placed on the blood and marrow pictures alone To further this end, clinical notes are incorporated in many of the legends A few words on treatment are frequently interspersed, especially where response to treatment has a bearing on diagnosis The general aim of the book is to promote accuracy in diagnosis

The recently standardized nomenclature is used, excepting that related to the erythrocytic series. Occasional references are made to the literature but, in view of the several encyclopedic books on hematology that are available, no formal bibliography is included.

The publishers imposed no restrictions on the size and number of illustrations, and were generous with respect to color. I availed myself of this opportunity to prepare photomicrographs of relatively large micro fields at high magnification, thus obtaining a better standard for comparison than the water-color drawings of blood and marrow cells that have been used so extensively in the past. Previous monographs and atlases of hematology have also limited their consideration of bone marrow almost exclusively to smears, sections of the tissue being rarely illustrated Sections are fully as important as smears, and are sometimes the only means of establishing a diagnosis. For this reason, I have given photomicrographs of sectioned marrow practically the same prominence as those of smears, and emphasize the fact that they should be used conjointly.

The material was accumulated as the result of special interest in hematology during my eighteen years as a hospital pathologist, first at the Philadelphia General Hospital and later at the Presbyterian Hospital in Philadelphia. The Hematology Clinic and wards of the Presbyterian Hospital afforded invaluable experience. Particularly rich were the war years when I was assigned to the Army Institute of Pathology in charge of the Division of Reticulo-Endothelial Pathology, where an unbelievable amount of material streamed in from Army and other sources Various colleagues generously contributed their cases to

certain gaps; for this help I am extremely ateful, and acknowledgment is made in the gend in each instance.

To Mr. Roy Reeve, master photomicrog-pher, and his staff at the Army Institute of thology goes my deepest appreciation. Mr. eeve personally took most of the pictures produced in the book, many of them difficult bjects that only one with his expert knowl-edge could have photographed at all well. To the series of medical technologists over the years, whose efforts provided me with good preparations for study, I owe a debt of thanks. No author could ask for greater patience, interest, and consideration than my publishers have shown.

R. PHILIP CUSTER

October, 1949

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Part I
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TERMINOLOGY

Many of the difficulties encountered in the field of hematology have been engendered by a complex and confusing nomenclature of cells and diseases of the blood and blood-forming organs. These series of terms have been built up over the years by individual observers and "schools," according to their own preferences, without thought of correlation and frequently in open antagonism to one another.

At the present time, the first serious move to clarify the situation is being made. A committee, sponsored by the American Society of Clinical Pathologists and the American Medical Association, has published a preliminary report of their deliberations on the question of leukocytes and thrombocytes* which is reprinted here practically in its entirety. I am in complete accord with their views, and have tried to conform to this recommended nomenclature wherever possible throughout the book.

Unfortunately, my illustrations were prepared prior to publication of the report, and some of the cell designations on the cuts are at slight variance with preferred terms, this does not assume serious proportions, however.

Clarification and definition of terms is urgently needed for the sake of a common understanding in clinical usage and in teaching of medical students and technicians. The choice of a preferred term, it was agreed, should not be based merely on historical priority or common usage but, in general, should represent the simplest, clearest, and most descriptive term. Eponyms and new terms should be avoided, wherever possible, without sacrifice of clarity. An effort should be made to attain consistency between related terms.

* Am J Clin Path 18 443, 1948

The various series of cells were considered. It was recommended that in Table 1 the term listed at the left replace all terms listed at the right in referring to cells of a particular series or to a disease affecting any cell of that series.

TABLE 1

Recommended Terms and Terms to Be Avoided When Referring to Cells of a Particular Series or to a Disease Affecting Any Cell of that Series

Term to Be Used	*Terms to Be Avoided*
Lymphocytic	Lymphoid, lymphatic, lymphogenous, lymphocyte, mononuclear
Granulocytic	Myeloid, myelogenous, myelocyte, myelocytic, granulocyte, leukocyte, leukocytic, leucocyte, leucocytic
Monocytic	Monocytoid, monocytogenous, mononuclear, monocyte
Plasmacytic	Plasma cellular, plasmacytogenous, myeloma cell, plasmacyte
Erythrocytic	Erythroid, erythrocytoid, erythron, erythrocytogenous, erythrocyte
Thrombocytic	Megakaryocytic, platelet, thrombocyte

[illegible]

histochemistry will contribute more clearcut criteria than are available at present.

It is recommended that the term *leukocyte* be considered synonymous with white blood corpuscle and include all white cells of the blood and their precursors in the blood-forming organs. Its use should not be limited to cells of the granulocytic series, excluding cells of the lymphocytic, monocytic or plasmacytic series. This and

other words derived from the same root should be spelled with a *k* and not a *c*, e.g., leukocyte, leukemia, not leucocyte or leucemia.

It is recommended that the descriptive terms for granules, *neutrophil*, *eosinophil*, *basophil*, and *azurophil* be spelled as indicated without a final *e*.

It is suggested that the name of the most undifferentiated of the cells of each series carry the suffix *-blast*, the second stage the prefix *pro-* and, except in the granulocytic series, all cells that are more mature than the -blast stage have names with the suffix *-cyte*. The name for the fourth stage in the granulocytic and erythrocytic series is to have the prefix *meta-*. The terms *blast cells* and *pro cells* may be used to replace other terms for these stages of development when speaking of the stage of development as a whole or when the series to which the cells belong has not been identified.

It is recognized that the *blast cells* of each series are morphologically very similar, all having fine nuclear chromatin structure, usually demonstrable nucleoli, and basophilic cytoplasm, with or without azurophil granules, so the prefix to be used will, in many instances, depend on the identification of the *pro-* stage associated with them.

Fine chromatin structure is defined as having the nuclear appearance of a background of homogeneous lighter-staining parachromatin, overlaid by a darker-staining lace-net meshwork or finely stippled pattern of basichromatin, with no aggregation of the basichromatin into even a single clump of appreciable size staining darker than any other areas in the nucleus.

A *nucleolus* is defined as a homogeneous blue-staining area within the nucleus of a cell, which stains more like the cytoplasm than does any other part of the nucleus.

The term *azurophil* should be applied to the granules seen typically in the cytoplasm of cells of the lymphocytic and monocytic series and the progranulocyte stage of the granulocytic series. The term *azurophil* is recommended, and not *azure*, in describing these granules, since the term refers to an affinity for a particular dye and not to the color of the granules. These granules may be present or absent in any cell of the lymphocytic series and when present are usually coarse and in clumps. They are usually present in all cells of the monocytic series, including the monoblast. In the monocytic series, they are usually fine, diffusely and uniformly scattered through the cytoplasm. If not seen in the monocyte or promonocyte, it usually indicates a faulty stain or poor visual definition in the microscope. These granules may be present or absent in any cell of the granulocytic series. They are rarely seen beyond the myelocyte stage except in disease. They are occasionally present in the cytoplasm of cells of the plasmacytic and erythrocytic series, and constantly present in the cells beyond the blast stage in the thrombocytic series where they tend to be fine and few in the early stages and numerous and often clumped in the more mature stages.

TABLE 2

RECOMMENDED TERMS AND TERMS TO BE AVOIDED WHEN REFERRING TO SPECIFIC CELLS OF THE BLOOD AND BLOOD-FORMING ORGANS

Name of Series	*Term to Be Used*	*Terms to Be Avoided*
Lymphocytic	Lymphoblast	Myeloblast, hemocytoblast, lymphoidocyte, stem cell, lymphocyte
	Prolymphocyte	Large lymphocyte, pathologic large lymphocyte, atypical leukocytoid lymphocyte, monocyte, immature lymphocyte
	Lymphocyte	Small, medium or large lymphocyte, normal lymphocyte, small, medium or large mononuclear
Monocytic	Monoblast	Myeloblast, hemocytoblast, lymphoidocyte, lymphocyte, stem cell, immature monocyte
	Promonocyte	Premonocyte, hemohistioblast, immature monocyte, Ferrata cell
	Monocyte	Large mononuclear, transitional, clasmatocyte, endothelial leukocyte, histiocyte, resting wandering cell
Granulocytic	Myeloblast	Granuloblast, hemocytoblast, lymphoidocyte, lymphocyte, stem cell
	Progranulocyte	Promyelocyte II, leukoblast, myeloblast, premyelocyte, promyelocyte, progranulocyte A
	Myelocyte	Granulocyte, myelocyte B, non-filament, class I
	Metamyelocyte	Metagranulocyte, juvenile, myelocyte C, non-filament, class I

TABLE 2 (*continued*)

Name of Series	*Term to Be Used*	*Terms to Be Avoided*
	Band cell	Staff cell, stab cell, non-filament, class I, rod nuclear, polymorphonuclear, stabkernige, rhabdocyte, non-segmented
	Segmented	Polymorphonuclear, filamented, class II, III, IV, or V, lobocyte
Plasmacytic	Plasmablast	Myeloblast, hemocytoblast, lymphoidocyte, lymphocyte, stem cell, lymphoblastic plasma cell, myeloma cell
	Proplasmacyte	Turk cell, Turk irritation form, lymphoblastic or myeloblastic plasma cell, myeloma cell
	Plasmacyte	Plasma cell, Unna's plasma cell, Marschalko's plasma cell, plasmacytoid lymphocyte, myeloma cell
Thrombocytic	Megakaryoblast	Megalokaryoblast
	Promegakaryocyte	*Premegalokaryocyte*
	Megakaryocyte	Megalokaryocyte
	Thrombocyte	Platelet, thromboplastid
——	Disintegrated cell	Senile cell, smudge, basket cell, smear cell, degenerated cell

It is recognized that in each cell series there is a continuous development from the most undifferentiated to the most differentiated stage, that an infinite number of subdivisions are possible, and that any subdivision is arbitrary. The committee recommended the use of the minimum number of subdivisions which will provide essential information for diagnostic and prognostic purposes and defined the lines of division between these stages as clearly as possible, basing these divisions on a single easily identifiable feature. As far as possible, the feature selected to differentiate the different stages of development is one which could be recognized in either stained or supravital preparations, but it is realized that at present the majority of such decisions will be based on smears stained with Wright's stain or with one of the other Romanowsky stains. Even with these definitions, cells will be encountered where decision is difficult, in which case it is suggested that the cell be arbitrarily placed in the more differentiated category.

Names were selected for each of the cells, which were acceptable to all members present and which, in the opinion of the committee, were least likely to be confusing.

The recommended terms and the terms to be avoided are listed in Table 2.

[illegible]

It is recognized that to ensure flexibility and for certain specialized purposes finer subdivisions may be necessary than those herein recommended. It is suggested that in such case no change be made in the term or definition of the recommended major divisions but that *clearly de-* [illegible] together with the suggested term, should be submitted for consideration by a permanent body which it is hoped will develop out of this committee.

The definitions decided on are as follows.

Lymphoblast. Any cell of the lymphocytic series having fine chromatin structure in the nucleus. Cells of blast morphology associated with prolymphocytes should be tentatively classified as lymphoblasts.

Prolymphocyte. Any cell of the lymphocytic series intermediate in morphology between the lymphoblast and the lymphocyte. It will always have too coarse a chromatin structure to fit the criteria for a blast and too fine a chromatin structure or too large a cell diameter to be classed as a lymphocyte. Usually, but not always, prolymphocytes are larger than 15 microns in diameter, which is the upper limit for the lymphocyte.

Lymphocyte. Any cell of the lymphocytic series having the morphology of those commonly found in the blood of healthy adults.

Monoblast. Any cell of the monocytic series having fine chromatin structure. Usually nucleoli are visible. Cells of blast morphology found in association with promonocytes should be tentatively classed as monoblasts.

Promonocyte. Any cell intermediate in morphology between the monoblast and the monocyte. It is differentiated from the monoblast by having an irregularly shaped nucleus and somewhat coarser chromatin structure, and from the monocyte by the presence of one or more nucleoli.

Monocyte. Any cell of the monocytic series having the morphology of those commonly found in the blood of healthy adults. It is differentiated from the promonocyte by the absence of nucleoli.

Myeloblast. Any cell of the granulocytic series having fine chromatin structure and containing no specific granules. Usually nucleoli are visible. Cells of blast morphology found in association with progranulocytes should tentatively be classed as myeloblasts.

Progranulocyte. Any cell of the granulocytic series which has a nuclear structure too coarse for that of a blast cell and which has not yet developed discernible, specific granules. This term was selected rather than "promyelocyte" because of its clear relationship to the definition of granulocyte, given below, and because the term "promyelocyte" has been in wide use for cells which do contain specific granules. The reason that the terms "granuloblast," "granulocyte," and "metagranulocyte" were not chosen was that the terms "myeloblast" and "myelocyte" were already in general use with essentially the definitions here given. This is true also for the term "granulocyte," which would otherwise have to be synonymous with the term "myelocyte."

Specific granules. Neutrophilic, eosinophilic or basophilic granules. This term does not include azurophilic granules.

Granulocyte. An inclusive term to apply to any cell containing specific granules. The plural form *granulocytes* would therefore include all myelocytes, metamyelocytes, band cells and segmented cells whether neutrophils, eosinophils, or basophils.

Myelocyte. Any cell containing specific granules, with a round or oval nucleus. It is distinguished from the progranulocyte by the presence of specific granules and from the metamyelocyte by the absence of indentation in the nucleus. It may be further subdivided, at the option of the user, into early and late stages, but the definition of early or late should be clearly stated in any publication.

This and all subsequent cells of the granulocytic series should be additionally characterized as neutrophil, eosinophil or basophil.

Metamyelocyte. Any cell of the granulocytic series having specific granules in the cytoplasm and a nucleus intermediate in shape between that of the myelocyte and the band cell. The nucleus usually has an indented oval shape, resembling a bean or kidney.

Band cell. Any cell of the granulocytic series which has a nucleus that could be described as a curved or coiled band, no matter how marked the indentation, if it does not completely segment the nucleus into lobes connected by a filament. It is differentiated from the metamyelocyte by an appreciable length of the nucleus having parallel sides, and from the segmented neutrophil by having no indentation which could be described as a filament.

Segmented cell. Any cell containing specific granules in which the lobes of the nucleus are connected by a filament. A *filament* is defined as a threadlike structure. Since at times, in viewing a three-dimensional object from one direction, it is impossible to be certain whether two parts of the nucleus are connected by a filament or band, it is suggested that such cells always be placed in the segmented category, since this is the more differentiated and more common cell.

The term *toxic neutrophils*, followed by a 1 to 4+ designation, is recommended for the grading of toxic granules, basophilia of the cytoplasm, vacuoles and condensation of nuclear chromatin in the neutrophils, since its meaning is clear, although it is recognized that it is not an adequately descriptive term. The grading should depend more on the degree of change than on the percentage of the cells involved and should be recorded in the report whenever the degree of change exceeds 2+.

Plasmablast. Any cell of the plasmacytic series having fine chromatin structure in the nucleus. Cells of blast morphology found in association with proplasmacytes are usually seen only in plasmacytic leukemia or multiple myeloma. The cytoplasm tends to be more opaque in staining than in the other leukocytic blast cells.

Proplasmacyte. Any cell of the plasmacytic series with a nuclear structure too coarse for that of a blast cell but with one or more nucleoli present.

Plasmacyte. A cell characterized by extremely coarse chromatin structure, with the deeply staining chromatin of the nucleus aggregated into large, sharply demarcated clumps. It is differentiated from the proplasmacyte by the absence of nucleoli. The cytoplasm of all cells of the plasmacytic series tends to be deeply basophilic and opaque in appearance. Azurophil granules may be present or absent, but are more commonly absent.

Megakaryoblast. Any cell of the thrombocytic series having a nucleus with fine chromatin structure. Usually these are larger than the other blast cells.

Promegakaryocyte. Any cell of the thrombocytic series with a nucleus containing nucleoli but having a chromatin structure too coarse for a blast cell. The nucleus is usually similar in shape to that of the megakaryocyte. Fine azurophilic granules are usually diffusely scattered through the cytoplasm.

Megakaryocyte. Any nucleated cell of the thrombocytic series in which nucleoli are not discernible. The azurophilic granules are often aggregated into clumps. Megakaryocytes and promegakaryocytes are typically much larger than other cells found in the marrow.

Thrombocyte. Any cell of the thrombocytic series containing no nucleus, in other words, any non-nucleated fragment of megakaryocytic cytoplasm containing azurophilic granules similar to those of the mature megakaryocyte.

The term *thromboplastid* was recognized as being anatomically correct, but it was felt that to be consistent with the use of the term *erythrocyte* and to permit the use of "thrombocytic" and "erythrocytic" in describing these cell series, the suffix "cyte" was preferable for these two non-nucleated forms.

Disintegrated cell. Any cell of any series in which the cytoplasmic outline has been disrupted or the nuclear chromatin is no longer surrounded by a membrane, excluding the changes in the nucleus that occur in mitotic division. Disintegrated cells should be recorded as such

in the differential report, even though they could be identified by dispersed granules They should be counted even if only shreds of nuclear material are discernible, since they are undoubtedly included in the total leukocyte count

It is, of course, understood that modifying adjectives may be applied to any of the recommended terms in describing results of investigation, but if these terms are to gain general acceptance they should not be given any new definitions except by general action of the committee

A subsequent report of the Committee on Nomenclature designates the successive developmental stages in the erythrocytic series as follows: *rubriblast*, *prorubricyte*, *rubricyte*, *metarubricyte*, *reticulocyte*, and *erythrocyte*, with the qualifying adjective phrase, *pernicious anemia type*, when used in connection with this family of diseases The first four of these terms are at sufficient variance with those commonly employed at present that they will not be used in this book. Should they become generally adopted, substitutions will be made in any later edition. Consequently, I have tabulated several series of terms employed to designate the various developmental stages in this series. The terms on the left of Table 3 are used in this book as listed in Fig. 12. The interrelationship of normal and abnormal erythropoiesis during the remission phase of pernicious and allied anemias is mentioned in the discussion of pernicious anemia (p. 59).

With respect to cells of the *reticulo-endothelial system*, those occupying an interstitial position are referred to as *reticulum cells*, those in the periphery of small vessels as *adventitial cells*, and

TABLE [illegible]

TERMS USED WHEN REFERRING TO [illegible] CELLS OF THE ERYTHROCYTIC SERIES

Normal Erythropoiesis		*Abnormal Erythropoiesis*
THIS BOOK	OTHER	
Procrythroblast	Pronormoblast	Pr[illegible]blast
Early erythroblast (basophilic)	Basophilic normo[illegible]	Basophil[illegible]
Intermediate erythroblast (polychromatophilic)	[illegible]	[illegible]
Late erythr[illegible] (acidop[illegible])	[illegible]	[illegible]
Norm[illegible]	[illegible]	[illegible]
Reticul[illegible]	[illegible]	[illegible]
Erythrocyte	[illegible]	[illegible]

those lining [illegible] *endothelial* [illegible] located [illegible] designations [illegible] undifferentiated [illegible] embryonal mesenchyme [illegible] phagocytic function they are most [illegible] *macrophages* or *histiocytes* (either fixed or wandering according to their position in the tissues). The so-called *epithelioid cell* is a morphologic variant of this group and probably occupies a position between the monocyte and macrophage.

Introduction. The remarkably constant numerical levels maintained by the cellular elements of the blood in healthy persons are the result of a continuous, precise balance between cell formation and destruction. This is all the more noteworthy when one realizes that under favorable conditions, the life span of erythrocytes is estimated at from 110 to 130 days, the span of leukocytes and thrombocytes is from three to five days, and replacements are constantly required. The tissues concerned with this mechanism, aptly termed the *hemolytopoietic system* by Krumbhaar, are the bone marrow, spleen, lymph nodes, liver, and stomach The manner in which each participates will be mentioned

Any deviation from normal cell levels in the circulating blood merely reflects an imbalance between blood formation and blood destruction or loss. Thus, a patient may be anemic by reason of inadequate erythropoiesis, excessive hemolysis or hemorrhage, or he may have an abnormally high red blood cell count when erythrocytes are produced at a rate in excess of their destruction. Likewise, leukopenia results from inhibited formation or maturation of leukocytes or their increased destruction or impaired delivery into the circulation. Leukocytosis occurs when there is a stimulus to overproduction by the parent tissues, purposeful in infections, destructive in the leukemias In much the same fashion, quantitative or qualitative changes in thrombocytes indicate altered formation or disposal.

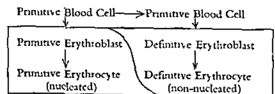
An intelligent consideration of peripheral blood changes must necessarily be based on a practical knowledge of the blood-forming and blood-destroying organs. The chapters that follow are planned to correlate alterations in the blood with those in the marrow.

EMBRYOGENESIS OF BLOOD AND RELATED CELLS

All formed elements of the blood take their origin from embryonal connective tissue, the *mesenchyme*, generally through *primitive blood cells*. In the primitive-streak and somite stages of the embryo, these cells are found exclusively in the yolk sac during the formation of blood islands in the area vasculosa, coincident with the development of the earliest blood vessels

Erythrocytes. The primitive blood cells proliferate rapidly, mainly within the lumina of newly formed vessels. The majority acquire hemoglobin, and thereby become *primitive erythrocytes*. These are large cells with abundant acidophilic cytoplasm, and most of them retain a small, compact nucleus (Fig. 1). They serve

disappear from the circulation. Other primitive blood cells which have not differentiated in this fashion reproduce themselves and about the sixth week, many begin to form *definitive erythroblasts* which mature to adult, non-nucleated erythrocytes (Fig. 2). These two distinct generations of erythrocytes may be more clearly illustrated as follows.



The curve indicates the regression of the first generation and the ultimate succession of the second.

Fig 1. *Primitive Erythrocytes* Cross-section of a blood vessel from a one-month embryo The first-generation erythrocytes are large, and the majority are nucleated They contain a rich complement of hemoglobin (× 750)

Fig 2 *Definitive Erythrocytes* Section through a blood vessel of a three-month fetus The red blood cells are all adult forms, and the admixed erythroblasts and normoblasts are also of the definitive type Figure 1, taken at the same magnification, shows the comparative size of the two generations (× 750)

Fig 3 *Hematopoiesis in Fetal Liver at Two Months.* The liver cells (L) are rendered inconspicuous by hematopoietic elements, virtually all red cell progenitors A few myelocytes (M) are seen Blood formation takes place almost exclusively outside of the sinusoids, a few sinusoidal lining cells (E) are visible (× 600)

Fig 4 *Hematopoiesis in Fetal Liver at Four Months* Liver cells (L) are hardly discernible in the mass of hematopoietic tissue which is still made up largely of megaloblasts (M), erythroblasts (E), and normoblasts (N) (× 600)

Fig 6 *Hematopoiesis in Fetal Liver at Six Months* Here and in Fig 5, erythropoiesis (E) is seen almost entirely within the liver lobules Cells of the granulocytic series (GR) are interspersed, but they appear most prominently in the connective tissue of portal triads (enclosed within the dotted perimeter) (× 600)

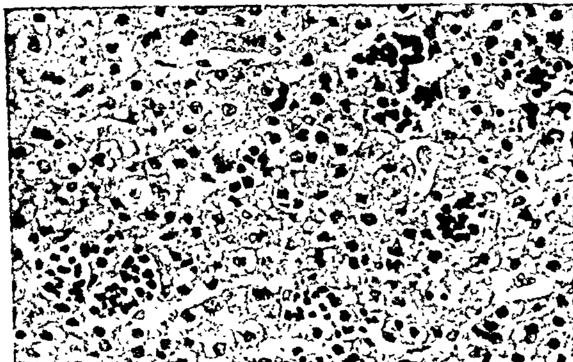
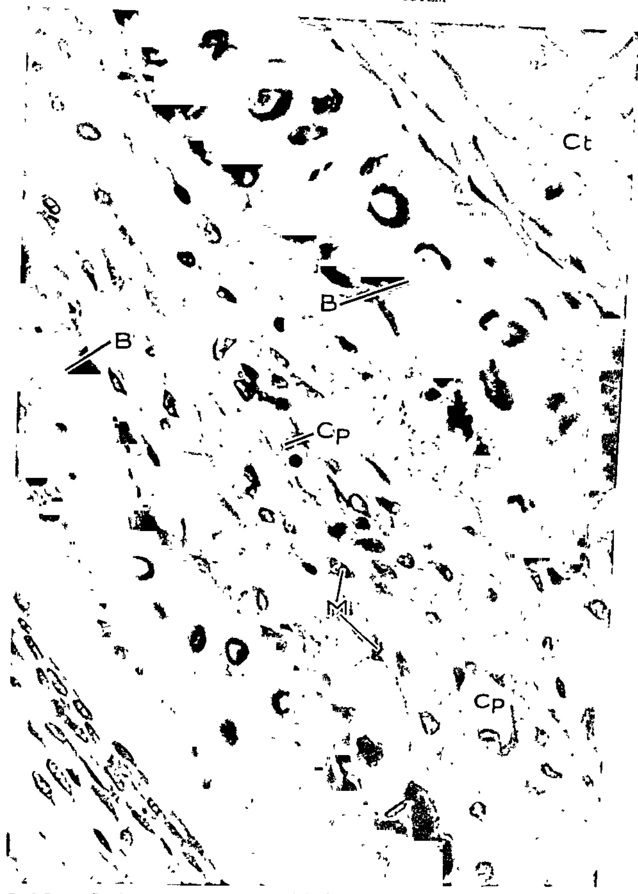


Fig 8 *Hematopoiesis in Fetal Liver at Nine Months.* Residual clusters of erythroblasts and normoblasts persist, mostly within sinusoids, as opposed to the almost strictly extravascular location noted in the early months At birth, a normal full-term infant displays very few such foci (× 600)


Ct
B
B
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M
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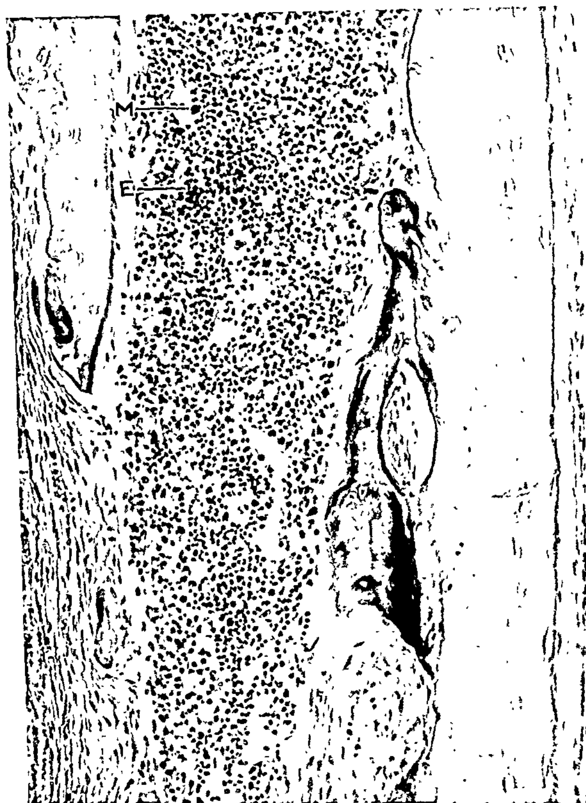


Fig 10 *Fetal Bone Marrow at Four Months* Longitudinal section through a rib discloses a well-developed marrow cavity filled with active hematopoietic tissue The bone cortex is imperfectly formed and no trabeculae have appeared Small islands of erythropoiesis (E) have sprung up and megakaryocytes (M) are occasionally seen, but for the most part, the marrow is devoted to production of cells of the granulocytic series (× 330)

Sites of Formation. LIVER. Blood formation in the yolk sac has virtually ceased by the ninth week, this function having been assumed mainly by the liver (Figs. 3 to 8), where evidences of hematopoiesis may be noted about the sixth week. Here it is extravascular, and red cell progenitors apparently arise from a thin layer of undifferentiated mesenchyme lying between the vascular endothelium of the sinusoids and the liver epithelium. Definitive erythroblasts proliferate so rapidly that by the fourth month they outnumber the liver cells. The mature erythrocytes thus formed slip through the delicate sinusoidal lining into the general circulation along with moderate numbers of erythroblasts and normoblasts

SPLEEN Between the third and sixth month, the spleen is engaged in erythropoiesis, but its role is relatively minor in comparison with that of the liver.

BONE MARROW Bone marrow, the permanent site of hematopoiesis, is formed between the second and fourth month, depending on the type of bone. Strands of vascularized mesenchyme grow into the developing bones and cause resorption of their centers. The spaces thus formed become the seat of active proliferation of the mesenchymal cells (Fig. 9), which soon differentiate to specific types of blood cells. By the fourth month, the marrow has become a rather effective blood-forming center (Fig. 10). At first it produces only granular leukocytes, but erythropoiesis becomes more and more active as it regresses in the liver and elsewhere. At birth, only a few clusters of erythroblasts and normoblasts are found in the liver.

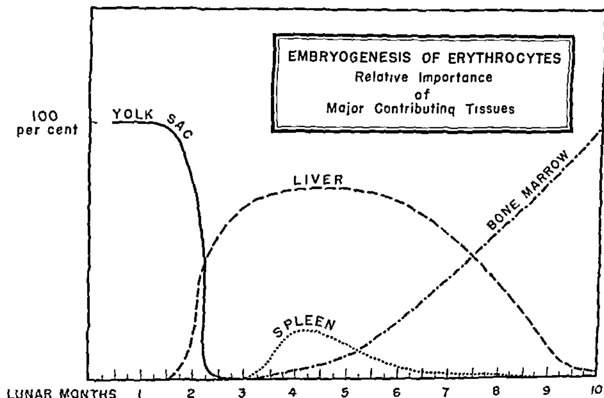


Fig 11 *Embryogenesis of Erythrocytes* The curves indicate in general the comparative participation of more important centers of erythropoiesis during antenatal life

THYMUS AND LYMPH NODES. Occasional clumps of definitive erythroblasts are found in the thymus and lymph nodes, but these are *never* important centers of red blood cell formation. Small foci may also be found outside hematopoietic tissues proper Figure 11 summarizes embryonal and fetal erythropoiesis

Leukocytes A few *myelocytes and wandering histiocytes* may be noted in blood islands during the early yolk sac stages of hematopoiesis The histiocytes, often actively phagocytic, are found in both intravascular and extravascular positions, and appear to develop either from

primitive blood cells or directly from mesenchyme. Cells of the *granulocytic series* are relatively inconspicuous during the first month or two, but become more evident as blood formation progresses in the liver, mainly in the interstices and especially around the portal triads With the advent of hematopoiesis in the bone marrow, granulopoiesis becomes and remains the dominant process, although giving way in part to formation of red blood cell progenitors as erythropoiesis in the liver recedes Scattered clusters of granular leukocytes, especially eosinophils, may be found in various other tissues during the later months, especially in the spleen, lymph nodes, and thymus. The spleen, lymph nodes, and other aggregates of lymphoid tissue are the main sites of *lymphocyte* formation. Like other blood elements, these cells are derived from the mesenchyme and pass through a primitive, undifferentiated stage. Lymphocytes analogous to adult forms are not in evidence until the third or fourth month of fetal life. *Monocytes* appear between the fourth and fifth month and are formed largely in the spleen and lymph nodes.

Megakaryocytes Giant cells resembling megakaryocytes, arising apparently from primitive blood cells, have been observed in blood islands of the yolk sac. Later, definite megakaryocytes appear coincident with active hematopoiesis in various organs, notably the liver, bone marrow, and spleen They usually lie extravascularly and may develop directly from mesenchyme, as well as through free primitive cells

The Reticulo-endothelial System. With the maturation of organs and tissues, the fixed mesenchymal cells fall into relative obscurity but retain their totipotential qualities throughout life, particularly as regards hematopoiesis. They remain the stem cells of the blood. They lie in intimate relation with the delicate argyrophilic reticular fibrillae of the tissue framework, and are found as stellate cells in the interstices (*reticulum cells*). Other similar but more flattened forms line blood and lymph sinuses (*specialized endothelial cells*, to be distinguished from adult vascular lining endothelium). Cells of this system are most abundant in the bone marrow, spleen and lymph nodes, and include Kupffer cells of the liver. They are also distributed throughout the body, especially in the adventitia of small vessels.

Reticulo-endothelial cells are distinguished by their remarkable phagocytic property. They are fixed histiocytes (tissue macrophages), they have the ability to transform into free ameboid forms (wandering histiocytes, wandering macrophages), and to revert to their original fixed tissue positions.

POSTNATAL HEMOLYTOPOIESIS

Theories of Blood Cell Origin Several theories have been advanced regarding development of the various blood cells during postnatal life, each admitting, however, a common origin in the reticulo-endothelial system (the adult counterpart of embryonal mesenchyme) Proponents of the *monophyletic theory* recognize a free intermediary multipotential primitive cell from which all types of blood cells arise in response to general or environmental requirements. The *polyphyleticists* state that the free blast cells are already differentiated as far as their capacity of developing toward a specific cell type is concerned Some also maintain that erythropoiesis is strictly intravascular, whereas leukopoiesis occurs mainly outside vessels

It is not within the scope of this book to discuss the relative merits of these theories. The developmental scheme presented in Fig. 12 represents a compromise that has proved practical.

Erythropoiesis Erythropoiesis is normally limited to the red bone marrow. The sequential steps are shown diagrammatically in Fig. 12. The composite cell group is generally known as the *erythrocytic series* In normal bone marrow preparations, proerythroblasts are rarely if ever encountered, and early erythroblasts are relatively few. Intermediate and late erythroblasts, and normoblasts are found in increasing numbers in the order named, virtually in geometric progression Mitotic division does not occur beyond the late erythroblast stage.

Orderly maturation in the erythrocytic series and the production of normal erythrocytes depend on adequate amounts of certain substances, such as the hematopoietic principle,

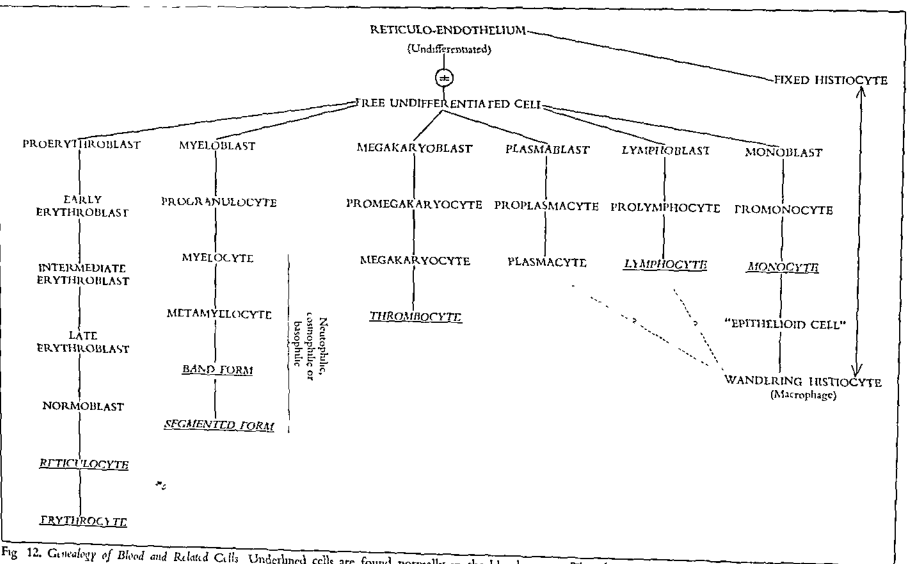


Fig 12. *Genealogy of Blood and Related Cells* Underlined cells are found normally in the blood stream The plus-minus mark (±) implies that development may take place through a free undifferentiated cell, or that differentiation may occur directly from the fixed stem cell.

iron, traces of copper, manganese or other metals, vitamins (especially vitamin B complex), and internal secretions (especially thyroxin).

The *hematopoietic principle* was generally thought to result from interaction of an extrinsic factor in the diet and an intrinsic factor secreted by the stomach, the product being absorbed from the intestine, stored mainly in the liver, and withdrawn as required for maturation of erythrocyte progenitors The isolation of vitamin B_{12}, a substance containing cobalt, and subsequent clinical observations on its therapeutic effects, indicate that vitamin B_{12} may be the hematopoietic principle per se, and that the intrinsic factor may merely promote its absorption. The need for iron in the synthesis of hemoglobin is self-evident. Copper and other metals serve merely as catalysts in the utilization of iron Thyroxin is thought to maintain a proper metabolic level.

Granulopoiesis. The red bone marrow is practically the exclusive site of granulopoiesis. Proliferation of progranulocytes and myelocytes serves to supply the usual daily replacements in the blood, although activity on the part of myeloblasts is often observed in normal marrow. Mitosis ceases with the fully developed myelocyte, and the cells thus formed go on to maturation, the nuclei becoming indented, then pyknotic, and finally lobate with filamentous interlobar strands.

Eosinophils and basophils are more likely to be formed outside the bone marrow than are neutrophils, eosinophils especially in lymphatic tissues in children A rather pronounced complement of eosinophils has been noted in the spleen of adults in cases of sudden death, indicating that they are usual residents of that organ. Tissue basophils (mast cells) are normal components of the skin, thymus, uterus, and breast, evidently formed locally from reticulum cells.

Lymphopoiesis. The spleen, lymph nodes, and the lymphatic tissue elsewhere produce cells of the lymphocytic series In common with other blood cells, they spring from undifferentiated reticulum cells and pass through a definitive maturation cycle. The genetic function of the so-called "germinal centers" of lymphatic tissue has not been clearly established, and under normal circumstances, lymphoblasts are not prominent Variation in size is probably not an index of relative maturity of the cells

Plasmacyte Formation. Some observers regard plasmacytes as offshoots of the lymphocytic series There is perhaps better evidence that they have their own genetic line and that a plasmablast is a distinct cell entity. Plasmacytes rarely appear in the peripheral blood under normal circumstances, but are found in lymphatic and interstitial tissue. When they proliferate after an inflammatory stimulus, their origin from reticulum cells becomes apparent. The same developmental series can be demonstrated in plasmacytic leukemia and in multiple myeloma *Russell body cells*, which have brilliant acidophilic granules and masses in their cytoplasm, are probably altered plasmacytes

Monocyte Formation. These cells may arise at any point in the reticulo-endothelial system, but are formed in greatest numbers in the spleen and lymph nodes through monoblasts and promonocytes, either directly from the reticulo-endothelium or through a free, undifferentiated primitive cell They apparently may undergo transition to the so-called "*epithelioid cell*," which in turn admits of further change to the *macrophage* (wandering histiocyte). This last form, however, usually results from the rounding up of fixed histiocytes

Megakaryocyte and Thrombocyte Formation. It has been said that the megakaryocytes are formed by coalescence of reticular or endothelial cells. There is far more reason to believe, however, that they develop through a free primitive cell from reticulo-endothelial elements in much the same fashion as other cell types described.

Megakaryocytes are found largely in the red bone marrow, where their position along blood sinusoids is often demonstrable. In properly fixed material, tiny pseudopods may be seen extending into the lumen of the vessels where they presumably break off (see Fig. 144), this is thought by some observers to be the mechanism of thrombocyte delivery.

Megakaryocytes are also found in the lung; certain authors believe that they are formed there, and that this is the main site of thrombo-

cyte formation. It is virtually certain that these cells are carried to the lung by the blood and there disintegrate.

Blood Destruction. Effete *erythrocytes* are disposed of mainly through a process of fragmentation. This occurs in the blood stream proper,

Worn out *leukocytes* and *thrombocytes* are likewise taken up by the reticulo-endothelial system, although many leukocytes pass out in the saliva and along the mucous membranes. Lymphocytes in particular are lost through the intestinal tract.

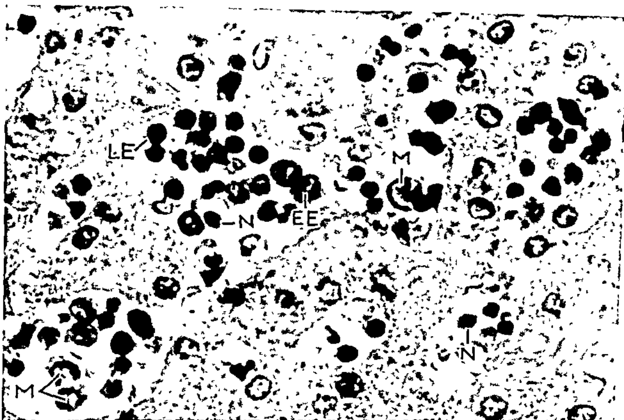


Fig 13 *Extramedullary Hematopoiesis, Liver.* This section is from a fourteen-year-old girl who died after five months of severe, continuous vaginal bleeding; the erythrocyte count having fallen to 700,000 per cu mm [illegible]

[illegible] gross description accompanied the small blocks of tissue submitted for examination, but it is assumed that both liver and spleen were enlarged The section of liver displays large numbers of erythrocyte progenitors, chiefly late erythroblasts (LE) and normoblasts (N), as well as early erythroblasts (EE) and a scattering of proerythroblasts (M) Except that erythropoiesis is chiefly intrasinusoidal, the appearances are similar to the fetal liver at about the seventh month (× 1000)

creating a fine hemoglobiniferous dust which is withdrawn from circulation by phagocytic cells of the reticulo-endothelial system. The breakdown of the hemoglobin molecule within these cells probably begins with separation of the protein, globin, from the iron-containing fraction, hemochromogen. Hemochromogen is oxidized to hematin, which undergoes further resolution to bilirubin and hemosiderin Bilirubin is excreted by the liver, most of the hemosiderin is re-used in the synthesis of hemoglobin

EXTRAMEDULLARY HEMATOPOIESIS

("Myeloid Metaplasia")

By definition, the term "extramedullary hematopoiesis" signifies blood formation in tissues other than bone marrow It is a compensatory mechanism, to be distinguished from purposeless ectopia of bone marrow, and represents a reversion of the involved tissues to [illegible] islands [illegible] preexisting cells of the reticulo-endothelial system

which retain their totipotential mesenchymal quality. The process occurs most commonly in those sites which were especially concerned with blood formation during intra-uterine life, as liver, spleen, and lymph nodes, but it is not necessarily restricted thereto.

The bone marrow space in infants and children is completely or largely occupied by hematopoietic tissue, whereas in adults, much has become fatty. Adults, therefore, have more room for expansion within the marrow proper when an undue burden is imposed on the blood-forming tissues, as by severe or protracted anemia. Thus, extramedullary hematopoiesis is noted more frequently in young people.

Causes of Extramedullary Hematopoiesis

Anemia. If anemia is of sufficient degree and duration, it is the most frequent cause of extramedullary blood formation. Even mild anemia of long standing may exhaust the reserve capacity of the marrow. The hematopoietic foci are generally composed of erythroblasts and normoblasts, although proerythroblasts may sometimes appear; the process is usually intravascular (Figs. 13 and 14). A scattering of myelocytes is often found in the adjacent interstices (Fig 14), and megakaryocytes may appear as well, either within or outside the vessels (Fig. 15).

Myelophthisic States When the marrow is largely replaced by tumor or fibrous tissue, myeloid metaplasia may attain tremendous proportions For example, a patient with widespread skeletal metastasis from a carcinoma of the breast had a spleen weighing 1100 gm, and its pulp was virtually indistinguishable histologically from bone marrow. In such instances, all the developmental series of the marrow participate extensively, here again, erythropoiesis is largely confined to the vessels, granulopoiesis to the interstices, and megakaryocyte formation occurs in either site.

Leukemias The so-called "leukemic infiltration" of the various tissues in leukemias is more likely to be an autochthonous process than a colonization of leukemic cells from the blood; reticulo-endothelial stem cells apparently proliferate along the pathologic developmental line.

In chronic granulocytic leukemia, there is often an associated metaplasia of erythrocyte progenitors and megakaryocytes in the spleen and elsewhere, and chronic lymphocytic leukemia may produce sufficient marrow displacement so that blood-forming centers spring up in other sites.

Extramedullary blood formation can be produced *experimentally* by repeated bleeding or by prolonged administration of cytotoxins, such as phenylhydrazine or sapotoxin. This has proved to be a valuable procedure in the study of blood cell origin.

ECTOPIC BONE MARROW

Strictly speaking, bone marrow ectopia is also extramedullary hematopoiesis, but it is considered separately because the ectopic tissue serves no purpose as an adjunct to an overworked bone marrow. It is merely the chance development of bone marrow outside the skeleton, sometimes as a congenital displacement (Fig. 16) or as part of a teratoid tumor (Fig. 17), but is more often associated with ossification of a calcific focus.

Tumor-like nodules of bone marrow unassociated with bone have been found in the paravertebral region of the thorax and abdomen, sometimes bilaterally symmetrical; they also occur in the adrenal cortex and about the renal pelvis. The development of marrow in ossified arterial plaques and cardiac valves, pulmonary scars, phthisical eyes, and many other sites is well known. Marrow emboli may lodge in branches of the pulmonary artery and become implanted.

The ectopic marrow may be either completely fatty or composed largely of blood-forming tissue. In the former instance, the unossified masses have been mistaken for simple lipomas.

Fig 14 *Extramedullary Hematopoiesis, Spleen* (Same case as Fig 13) There is an even greater degree of blood formation in the spleen, with erythropoiesis taking place mainly within blood sinuses The lining endothelium (End) is swollen and appears to be budding, this sinus endothelium may possibly be the source of the metaplastic foci. Myelocytes (My) are noted in the red pulp (× 1000).

Fig 15 *Extramedullary Hematopoiesis, Spleen* A metaplastic megakaryocyte is seen on the right, and a cluster of nucleated red blood cells lies at the left of the picture (× 1000)

Fig 16 *Ectopic Bone Marrow* Two symmetrical spheroid masses, 3 cm in diameter, were found beneath the pleura, one on each side of the body of the fifth dorsal vertebra, in an elderly woman who had died of arteriosclerotic heart disease The masses were encapsulated and separate from the vertebra and the underlying ribs Sections disclosed normal, active bone marrow, considerably more cellular than marrow in the adjacent vertebra (× 350)

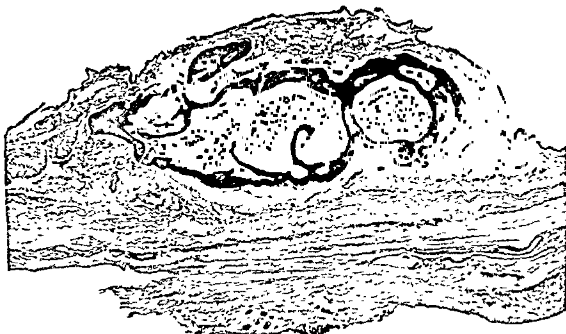


Fig 17 *Ectopic Bone Marrow* A portion of the wall of a typical ovarian dermoid cyst contains a plaque of bone, the marrow spaces of which contain actively hematopoietic tissue and admixed fat

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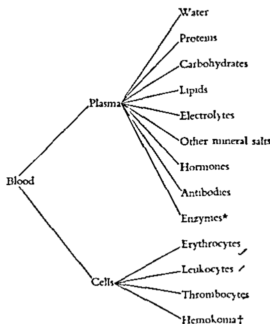
NORMAL BLOOD AND BONE MARROW

BLOOD CONSTITUENTS

The total blood volume at birth averages about 300 ml. It doubles during the first year, and increases gradually thereafter, having a closer relationship to body weight and surface area than to age. The blood comprises about 6 to 8

rific gravity ranges from 1.048 to 1.066 (serum, 1.026 to 1.031; erythrocytes, 1.092 to 1 095). It is slightly higher in males than in females and shows a minor diurnal variation.

Considering the blood as a tissue, this diagram reduces its components to a simple form:



* Derived partly from leukocytes.

† So-called "blood dust," particles about the size of neutrophil granules, visible in wet, unstained coverslip preparations of blood.

Figure 18 shows the cellular constituents of normal adult blood.

Erythrocytes. Knoll's figure of 366,900 red blood cells per cubic millimeter in embryos of 6 5 to 8 mm. appears to be the earliest count recorded, 92 per cent of the cells being nucleated primitive erythrocytes. Judging from cell size, however, the oxygen-carrying capacity of these cells is greater than that of the definitive erythrocytes which have completely replaced them by the fourth month (compare Figs. 1 and 2 of same magnification). Values for the later periods of intra-uterine life (Wintrobe) are shown in Table 4.

The blood *at birth* is characterized by macrocytosis (red blood cell diameter, 8 to 9μ), from slight to moderate polycythemia (5,000,000 to 6,000,000 per cu. mm), mild reticulocytosis (2 to 3 per cent), and the appearance of a few erythroblasts and normoblasts. A rather rapid fall in erythrocytes and especially in hemoglobin follows, accompanied by a reduction in cell size. By the third to fifth month, a microcytic anemia exists; the smaller cells, however, have resumed a normal complement of hemoglobin per unit volume. From this point, there is a gradual transition in both sexes. Girls reach adult values *by puberty;* boys show a rapid rise after puberty to reach normal adult male levels.

The data compiled by Wintrobe from various sources (Table 5 and Fig. 19) furnish reliable standards of comparison, especially as regards *adult values.** There are certain conditioning influences, such as ascent to high altitudes, which cause an actual increase in red

* Table 5 does not show the reduction in cell diameter, often to 5μ, which occurs between the third and sixth month

cells, hemoglobin, and volume of packed cells. The redistribution of cells, as from exercise, cold baths, abdominal massage, or excitement, temporarily elevates the erythrocyte count. Women may show cyclic variations dependent on menstrual blood loss. The effects of dehydration or water retention are obvious. By far the most common cause of fluctuations in red blood cell, hemoglobin, and volumetric values in healthy persons is the error inherent in the technical methods employed.

This inconstancy holds true to some extent during the early weeks *after birth*, although most infants display a primary leukocytosis, reaching a peak of 15,000 to 20,000 per cu. mm. by the end of the first twenty-four hours and falling quickly to about 10,000 within a few days. Neutrophils comprise the bulk of this first rise, many of them immature forms. Within a month, however, neutrophils drift into the background as a result of relative and actual lymphocytosis which maintains the total count somewhat above the normal adult level for four or five years, occasionally much longer, there is a fairly wide range that must be regarded as normal. The instability of leukocytes during childhood exaggerates the effect of conditioning factors, such as exercise, emotions, heat, sunlight, and minor infections, which also can elevate the count in adults

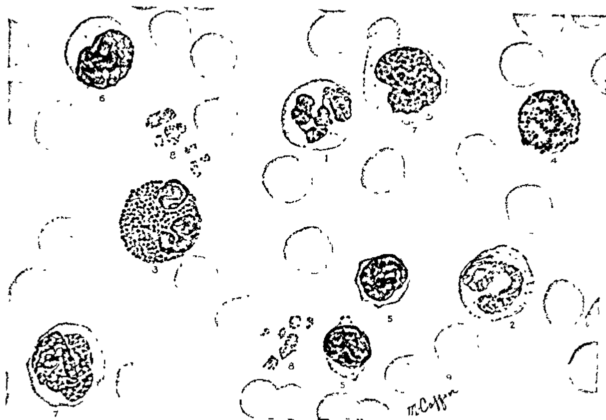


Fig 18 *Normal Cellular Constituents of Adult Human Blood* 1, Segmented (polymorphonuclear) neutrophil, 2, Band (stab) neutrophil, 3, Segmented eosinophil, 4, Basophil, 5, Small lymphocytes, 6, Large lymphocyte, 7, Monocytes, 8, Thrombocytes, and 9, Erythrocytes

Leukocytes. The blood of the early *embryo* is virtually devoid of leukocytes. By the second month, the count is said to be about 1000 per cu. mm , of which 25 per cent are recognizable as cells of the granulocytic series, the others being undifferentiated forms. Throughout *fetal life* the total leukocyte count may be variable, as indicated by Table 4, and the differential count does not follow any particular trend Lymphocytes of adult type do not appear until about the fourth month, and monocytes appear in the fifth.

Fluctuations decrease as puberty is passed; the *adult average* of 7000 to 8000 leukocytes per cu. mm. is established, and neutrophils become the predominating cell type, ranging from

60 to 70 per cent of the total. Table 6 presents the proportionate and absolute counts of the various leukocytes which are generally accepted as normal adult levels. Deviations are not uncommon, however.

TABLE 4

BLOOD VALUES IN 30 HUMAN FETUSES

Age (days)	*Fetus* Length Full (mm)	Length Cr.-R (mm)	Wt. (Gm)	R.B.C. × 10⁶	Hgb. (Gm)	Ht. (ml)	C.V. (c μ)	C.H. (γγ)	C.C. (%)	Icterus index	*R.B.C. Diameter* Fresh (μ)	Stained (μ)	*Immature Cells* N.R.C. (%)	Retics (%)	W.B.C. × 1000 (p c mm)
71	53	37	5.1	0.49	..	..	..	..	..		10.35	9.40	17.0	100	
72	57	43	5.7	0.49	..	.	..	.	.	.	8.41	7.34	3.0	87	7.8
78	..	..	8.7	0.31											
78	71	53	9.2	0.88	..	25.0	285	..	..	.	10.96	9.18	7.6	94	
88	..	..	..	1.82	..	.	136	..	..	15[a]	9.51	8.98	..	82	
95	..	88	..	0.95	8.0	22.8	249	93	36	7	..	7.86	0.3		
95	115	78	..	2.20	10.9	33.5	152	49	32	25[a]	9.90	8.45	1.4		
96	128	90	44	1.96	9.1	26.2	134	47	34	7	10.64	9.37	4.0	18	2.0
109	154	109	73	2.29	6.0	29.0	126	35	31	20	8.39	8.29	0.5	75	3.1
112	172	120	87	2.94	13.1	44.0	150	45	30	35	8.49	7.72	..	12	15.5
120	215	95	185	3.00	10.5	34.0	113	35	31	.	7.80	8.40	1.2	18	6.6
121	205	138	148	3.53	13.1	40.3	113	37	32	17[a]	9.56	7.62	.	19	12.2
126	222	155	..	2.29	10.9	36.2	154	48	31	20	8.85	7.70	0.9		
128	135	115	..	2.59	10.8	36.0	138	42	30	7	8.55	7.96	0.4	9	
131	220	150	245	2.46	10.2	32.5	132	42	32	30	..	7.54	0.6	18	11.4
133	218	160	255	2.24	11.3	32.0	139	51	36	.	8.30	7.90			
140	250	175	290	2.30	8.7	30.0	130	38	29	10	9.30	8.50	0.7	22	17.0
141	245	165	323	3.52	14.6	40.7	116	42	36	11	9.04	8.70	..	9	10.7
142	250	170	330	3.23	11.9	40.0	124	38	30	17	..	7.58	..	.	12.9
144	255	176	325	2.89	12.0	39.0	140	42	30	13	9.02	8.53	.	15	5.9
149	270	180	362	2.91	12.8	36.1	125	44	35	20	8.70	7.50	0.3	10	12.2
154	292	180	411	2.76	11.0	34.6	125	40	32	8	8.00	7.68	0.6	18	14.0
155	295	202	515	4.13	17.6	52.0	126	33	34	12	8.69	8.10	0.2	14	4.4
160	280	.	700	2.94	11.0	36.0	122	38	31	4	..	7.17	.	..	3.4
160	297	202	551	3.54	13.9	46.1	130	39	30	25	..	8.30	0.9	6	1.6
161	300	190	540	3.25	14.6	44.5	136	45	33	5	8.40	8.10	0.2	6	8.6
170	320	215	626	3.86	14.7	48.1	124	42	30	16	8.81	8.05	1.0	7	17.4
170	315	215	624	3.30	12.4	40.1	121	37	31	15	8.86	7.11	0.2	6	3.2
175	340	230	640	3.75	14.6	47.0	125	39	32	..	9.14	7.60	0.3	12	6.0
177	320	200	960	3.51	14.1	41.2	116	40	34	.	.	7.88	0.3	..	8.8

Ht = volume of packed cells per 100 cc blood, C.V = mean corpuscular volume, C.H. = mean corpuscular hemoglobin, C.C. = mean corpuscular hemoglobin concentration (From Wintrobe, M. M: Clinical Hematology. Lea & Febiger)

Neutrophils have a particular phagocytic affinity for bacteria and other minute particulate matter, and also elaborate a wide variety of enzymes, and possibly proteins and agglutinins. The chemotactic influence of bacteria on these cells is well established. The function of *eosinophils*, however, is still a question; their prominence in allergic reactions has indicated that they have to do with protein decomposition and histamine. *Basophils* (mast leukocytes) also remain an enigma; they differ from the other cells of the granulocytic series in that the granules are oxidase-negative and water-soluble. *Tissue mast cells*, which have in common with mast leukocytes only the basophilic staining quality of the granules, may possibly play a part in heparin formation. Recent work has suggested a relation of *lymphocytes* to production or transport of antibodies. *Monocytes* and the

TABLE 5

NORMAL VALUES FOR RED CORPUSCLES AT VARIOUS AGES

Age	Red Cell Count (millions per cu. mm)	Hemoglobin (gm per 100 cc)	Vol. Packed R.B C (ml per 100 cc)	Corpuscular Values			
				M C.V (c μ)	M C H (γγ)	M C H C (%)	M C D. (μ)
First day	5 1 ± 1 0	19 5 ± 5 0	54 0 ± 10 0	106	38	36	8 6
2–3 days	5.1	19 0	53 5	105	37	35	
4–8 days	5 1	18 3 ± 4 0	52 5	103	36	35	
9–13 days	5 0	16 5	49 0	98	33	34	
14–60 days	4.7 ± 0 9	14 0 ± 3.3	42 0 ± 7 0	90	30	33	8 1
3–5 months	4 5 ± 0 7	12 2 ± 2 3	36 0	80	27	34	7 7
6–11 months	4 6	11.8	35 5 ± 5 0	77	26	33	7 4
1 year	4 5	11 2	35 0	78	25	32	7 3
2 years	4 6	11.5	35 5	77	25	32	
3 years	4 5	12 5	36 0	80	27	35	7 4
4 years	4 6 ± 0 6	12 6	37 0	80	27	34	
5 years	4 6	12 6	37 0	80	27	34	
6–10 years	4 7	12 9	37 5	80	27	34	7.4
11–15 years	4 8	13 4	39 0	82	28	34	
Adults Females	4 8 ± 0 6	14 0 ± 2 0	42 0 ± 5 0	87 ± 5	29 ± 2	34 ± 2	7 5 ± 0 3
Males	5 4 ± 0.8	16 0 ± 2 0	47 0 ± 7 0	87 ± 5	29 ± 2	34 ± 2	7 5 ± 0 3

M C V. = mean corpuscular volume M C.H = mean corpuscular hemoglobin. M C.H C = mean corpuscular hemoglobin concentration M C D = mean corpuscular diameter (From Wintrobe, M M · Clinical Hematology. Lea & Febiger)

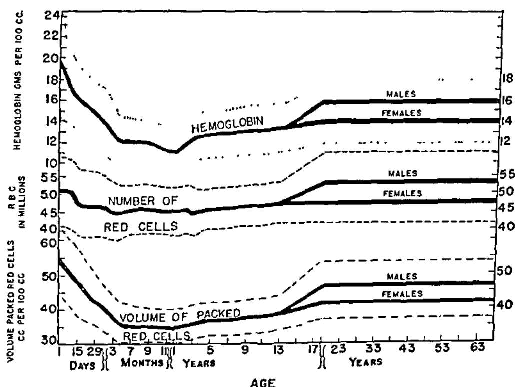


Fig 19 *Normal Curve for Hemoglobin, Red Cells and Volume of Packed Red Cells, from Birth to Old Age* The mean values are heavily outlined The range of variation is indicated by dotted lines for hemoglobin, interrupted lines for red cell count and dotted interrupted lines for volume of packed red cells The scales for hemoglobin, red cell count and volume of

Clinical Hematology Lea & Febiger)

closely related *wandering histiocytes* (macrophages) are avid phagocytes for products of tissue breakdown, foreign material, and bacteria; they are secondarily reacting cells in inflammation.

Finally, whereas red blood cells carry out their oxygen-transport function in the peripheral blood proper, leukocytes merely use the blood stream as a means of transportation to the tissues

foci in the proximal ends of the femora and humeri The disposition of fat follows growth of marrow spaces beyond the body requirements for blood-forming tissue. Temperature is probably the main factor in determining sites of marrow involution, the temperature of the extremities being appreciably lower than that of the torso and head. It has been shown experimentally that transplantation of the tip of a rat's tail into its abdominal cavity is followed by marrow hyperplasia of the implanted segments, while those segments that remain outside retain their fatty marrow.

TABLE 6

RELATIVE AND ABSOLUTE VALUES FOR LEUKOCYTE COUNTS IN NORMAL ADULTS PER CU MM BLOOD

Type of Cell	*Per cent*	*Absolute Number* *Average*	*Minimum*	*Maximum*
Total leukocytes	..	7,000	5,000	10,000
Myelocytes	0	0	0	0
Juvenile neutrophils	3–5	300	150	400
Segmented neutrophils	54–62	4,000	3,000	5,800
Eosinophils	1–3	200	50	250
Basophils	0–0 75	25	25	50
Lymphocytes	25–33	2,100	1,500	3,000
Monocytes	3–7	375	285	500

(From Wintrobe, M M . Clinical Hematology Lea & Febiger)

BONE MARROW CONSTITUENTS

Although widely dispersed, the bone marrow must be regarded as a single organ, the largest in the body. Its volume has been estimated as ranging from 67 to 91 ml. at birth and from 1320 to 4192 ml in adults. The total marrow space is greater in males than females, and the aging process in bone increases the volume in elderly persons.

At birth, the marrow cavity of all bones is completely filled with red (blood-forming) marrow. In early infancy, however, a few fat cells appear in bones of the extremities, and over the succeeding years there follows a gradual retrogression of hematopoietic tissue with concomitant fat replacement. The general trend of this process is indicated in Fig 20 By young adult life, the two long bones (tibia and femur) are virtually completely fatty, whereas the three flat bones (rib, sternum, and vertebra) retain their blood-forming function in varying degrees throughout life. The generalization may be made that, beyond the age of twenty, red marrow is limited to the vertebrae, sternum, ribs, clavicles, scapulas, and bones of the skull and pelvis, although there are also small

The bone marrow is the most labile tissue in the body, except the blood itself Fat may be displaced by spreading hematopoietic tissue in an extraordinarily short time. Transition from completely fatty to solidly cellular marrow within two days has been observed under experimental conditions. The process spreads distalward in general, so that bones of the forearms, legs, hands, and feet are late participants and undergo hyperplasia only as a result of most unusual stimuli, as in pernicious anemia or leukemia

Stroma and Nerves. The stroma of bone marrow is formed by a delicate framework of argyrophilic reticular fibers to which reticulum cells are closely applied. The fibers are attached to the endosteum and are closely connected with vascular walls. Nerves, largely sympathetic trunks, accompany the larger vessels (Fig 21). Relatively few sensory fibers are present, and ganglion cells have not been demonstrated.

Nutrient Vessels. These are sizable (Fig. 21), and are unusual in that they break up into a

vast sinusoidal network almost immediately on entering the bone The sinusoids are walled for the most part by a single layer of endothelium, but have an appreciably larger potential caliber than capillaries of other tissues. The volume of the combined lumina is such that the rigid bone casing makes it impossible for all to attain their maximum caliber at the same time.

There are apparently no lymphatic vessels in the marrow.

Cytologic Pattern. There is a considerable variation in the cellular components of bone marrow in presumably normal persons, even from bone to bone in the same person. In general, the hematopoietic tissue occupies areas between fat cells and open blood channels, the

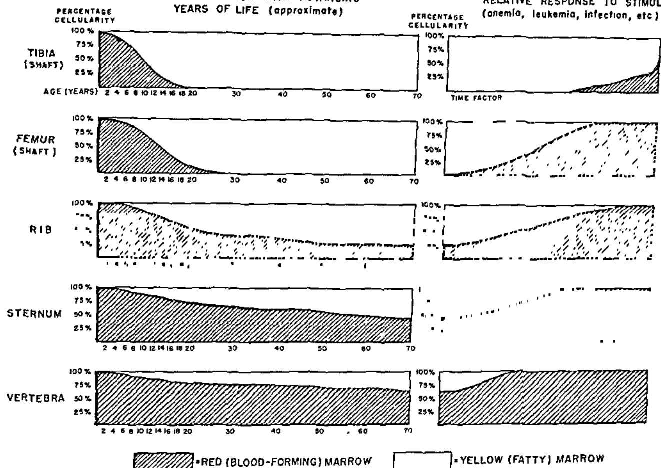


Fig 20. *Cellularity of Various Bones at Different Ages* The first section of this diagram represents the regression of hematopoietic tissue (with advancing age) and its replacement by fatty marrow in representative long and flat bones It is based on a series of autopsies on persons who died from causes unlikely to have affected the blood-forming organs

Many of the blood channels, therefore, are either reduced to fine capillary size or are closed to the circulation altogether. This unique vascular pattern lends support to the concept of Sabin, Doan, and others that the endothelium of closed intersinusoidal capillaries differentiates to form erythroblasts which mature within the lumina. The erythrocytes thus formed are said to be swept into the circulation during the intermittent opening of these channels

progenitors of cells of the granulocytic series forming a more or less diffuse background, and the nucleated red blood cells tending to aggregate in clumps. Megakaryocytes are relatively sparsely distributed and usually lie in juxtaposition to sinusoids; however, this relationship is not often apparent unless the marrow is studied by serial sections Cells of the reticuloendothelial system are inconspicuous



lie in the perivascular areolar tissue, relatively few sensory fibers are present (× 200)



Fig. 22. *Normal Sternal Bone Marrow* ... [illegible]

(progranulocyte), Mlc, myelocyte, Mlc', myelocyte in mitosis, Mmlc, metamyelocyte, St, stab (band cell), Eos, eosinophilic myelocyte, Mkc, megakaryocyte (uninuclear, these cells more characteristically have multilobate nuclei), Pl, platelets (thrombocytes) There are also a few lymphocytes intermingled, derived from admixed peripheral blood

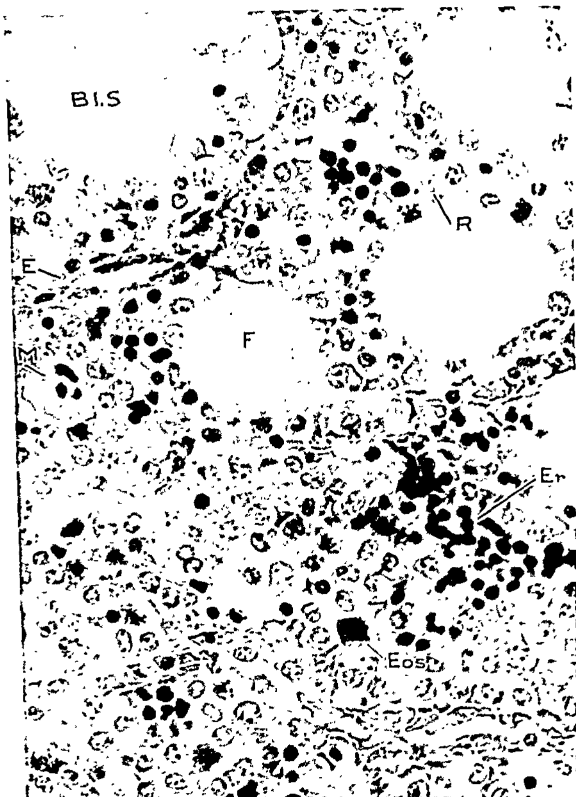


Fig 23 [illegible] without [illegible] occupied [illegible] nuclei be [illegible] (Eos) are conspicuous by their coarse granulation Reticulum cells (R) are scattered throughout the interstices and blood channels are lined for the most part by specialized sinus endothelial cells (E) Megakaryocytes (M) are sparsely distributed. Red blood cell formation tends to be focalized (Er), the majority of cells being in the erythroblastic and normoblastic stages (× 1000)

Small aggregates of lymphocytes are occasionally encountered in the marrow, especially in older persons.* The significance of these lymphoid nodules is not apparent. They are apt to be confused with the focal marrow involvement that one finds early in chronic lymphocytic leukemia

Erythrogranulocytic Ratio. The ratio may vary from 1·2 to 1:6 (it has been recorded as great as 1:10, but this seems rather too high to be regarded as normal). The erythrocytic and granulocytic series comprise from 90 to 95 per cent of the marrow cells. It is impossible to present a *normal differential count* as a standard for comparison, either from one's own material or from accounts in the literature. Methods differ far too widely, and counts on aspirated and sectioned marrow from the same person seldom correspond. The value of differential counts, unless performed on meticulously prepared marrow sections and carried out according to a standard technic, is seriously questioned. *When the deviations from normal exceed the variations shown in the table, it seldom requires a count to make them apparent.* Table 7 and Figs. 22 and 23 show what may be regarded as the usual finding in healthy adults.

The cellular state of fetal bone marrow has already been described as changing from a primarily pure granulopoietic tissue gradually to assume red cell and thrombocyte formation as well, so that *at birth* the erythrogranulocytic, ratio has been variously stated as 1 2 to 1.8. The pattern of the erythrocytic series differs somewhat from the permanent status, in that early and intermediate erythroblasts predominate over late erythroblasts and normoblasts, many procrythroblasts being present This same relative immaturity exists in the granulocytic series, so that myeloblasts and progranulocytes outnumber the later forms This is followed by a slow "shift to the right" during the succeeding months.

Cellular Output. The delivery of cells from the bone marrow is enormous. Even though somewhat less than half of the total capacity is operating under normal circumstances, it has been estimated that nearly one trillion erythrocytes, ten billion granular leukocytes, and five hundred billion thrombocytes pass into the blood stream daily.

TABLE 7

"NORMAL" DIFFERENTIAL COUNT*

Cell Type		*Range in Per Cent*
Undifferentiated Cells		0 0
Myeloblasts		0 0 – 3 5
Progranulocytes		0 5 – 5 0
Myelocytes	Neutrophil	7 0 – 34 6
	Eosinophil	0 3 – 3 0
	Basophil	0 0 – 0 5
Metamyelocytes and Band Cells	Neutrophil	14 8 – 33 0
	Eosinophil	0 3 – 3 7
	Basophil	0 0 – 0 3
Segmented Cells	Neutrophil	3 0 – 19 8
	Eosinophil	0 1 – 3 0
	Basophil	0 0 – 1 0
Procrythroblasts		0 0
Erythroblasts		4 2 – 18 2
Normoblasts		13 3 – 23 8
Megakaryoblasts		0 0 – 0 25
Megakaryocytes		0 05 – 1 2
Reticulo-endothelium		0 3 – 2 6
Monocytic series		0 1 – 3 2
Lymphocytes		0 0 – 6 8
Plasmacytes		0 0 – 1 2

* The figures in this table represent a compilation o counts made both from smears and sections of marrow Some samples were aspirated during life from persons in whom there proved to be no significant alteration in the tissue The others were obtained after accidental death or from patients dying of conditions such as brain tumor, who had normal blood counts The aspirates obtained during life naturally contained varying amounts of circulating blood, accounting for the occasionally high lymphocyte count

* Williams, R J Am J Path, *15* 377, 1939

Part II

Disorders of the Blood and Bone Marrow

CLASSIFICATION

Variations from normal blood are due either to primary disease of the blood-forming tissues or to the influence of some extraneous factor on blood formation, blood destruction, or blood loss Careful evaluation of the patient's history, symptom complex, physical examination, peripheral blood changes, and adjunct clinicopathologic studies frequently suffices to establish the diagnosis These data can be misleading, however, and direct examination of the bone marrow is often required to determine the cause of an obscure anemia, to explain a hemorrhagic state, or to differentiate leukemia from leukemoid reactions. Bone marrow biopsy in certain infectious diseases will sometimes disclose the offending organism when the clinical picture is an equivocal one. The cellular character of the marrow is quite specific in Gaucher's disease and related conditions Biopsy of the bone marrow has frequently disclosed primary or secondary tumors before they were evident on roentgenologic examination The finding of nonspecific hyperplasia or even a normal marrow, if not so dramatic, is fully as valuable in differential diagnosis.

One cannot emphasize too often or too strongly the need for precise diagnosis in the field of hematology Anemia is not a disease; it is a manifestation of something gone wrong, and the conscientious physician will employ all available means to find *what* has gone wrong Some busy practitioners find it far easier to prescribe "shotgun hematinics" than to perform a careful physical examination, and to call on the clinical pathologist and radiologist for help. Many patients who have a simple iron deficiency will respond well, although expensively, to the iron in these multiferous compounds. Likewise, the hypochromic anemia which follows chronic bleeding from carcinoma of the colon may be relieved for a time by the same means. To have the autopsy disclose a tumor that at one time could have been removed is good neither for one's conscience nor for one's practice.

Because etiology is so important in disorders of the blood-forming organs, the following classification is based mainly on cause, when known A certain amount of overlapping is inevitable in such a listing, but cross-reference will be made to avoid reduplication of descriptions and illustrations.

TABLE 8

CLASSIFICATION OF BLOOD DISORDERS

CHAPTER

- V. *Deficiency Anemias*
  - a General nutritional anemia
  - b Iron-deficiency anemias
  - c Deficiency of hematopoietic principle
  - d *Vitamin deficiencies*
  - e Endocrine imbalance
- VI Aplastic and Hypoplastic Anemias
  - a Primary
  - b Secondary
- VII Displacement of Bone Marrow
  - a Primary
  - b Secondary
- VIII Hypersplenism
  - a Primary
  - b Secondary
- IX Hemolytic Anemias
  - a Peculiar to infancy and childhood
  - b Familial and racial
  - c Acquired
  - d Paroxysmal hemoglobinuria
  - e Blood-transfusion reactions

- f. Infections
- g. Chemical agents
- h. Physical agents
- i Allergy
- j Malignant tumors

X Ill-Defined Anemias·
- a. Cachexia of malignant tumors
- b Chronic renal disease
- c. Banti's syndrome
- d. Anemias of pregnancy
- e Refractory anemias

XI Hemorrhagic States

XII Effects of Physical and Chemical Agents:
- a Heat
- b Cold
- c. Radiant energy
- d Chemical agents

XIII Leukocytosis, Leukemoid Reactions, Leukopenia

XIV. Infections·
- a Virus diseases
- b Rickettsial diseases
- c. Spirochetal diseases
- d Bacterial diseases
- e. Ill-defined conditions probably related to bacterial infection
- f Mycotic diseases
- g Protozoal diseases
- h Helminthic diseases
- i Bartonellosis and anaplasmosis

XV Leukemias·
- a. Stem cell (undifferentiated)
- b Granulocytic
- c. Lymphocytic
- d Monocytic
- e Plasmacytic
- f Thrombocytic
- g Leukemia in animals

XVI. Polycythemia.
- a Erythrocytosis (compensatory polycythemia)
- b Erythremia (polycythemia rubra vera)

The various conditions will be described in the order in which they appear in this classification

V

DEFICIENCY ANEMIAS

GENERAL NUTRITIONAL ANEMIA

(See also Nutritional Macrocytic Anemia, pp 60–61)

Anemia frequently results from an imbalanced or generally deficient food intake. This is more common in infancy and childhood, for example, from an unsupplemented milk intake, than in adults. Poverty, overenthusiastic reducing regimens, or starvation in anorexia nervosa may cause marked anemia even in adults. A similar situation may be encountered in advanced wasting diseases where the general nutritional status of the patient is at a low ebb.

Significant Laboratory Data The anemia is virtually always hypochromic and usually microcytic. There is often a wide range in red cell diameters, however, and poikilocytes are commonly seen. The red cells are thin, with hemoglobin forming a rim about the relatively empty center (Fig. 24). Slight to moderate

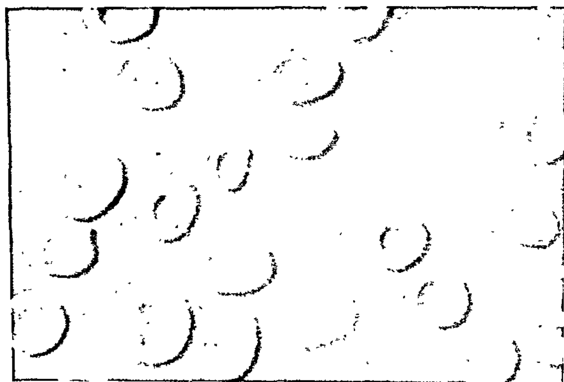


Fig 24 *General Nutritional Anemia Blood* Bas relief photomicrograph to emphasize the moderate degree of hypochromia The volume index was 1, but the blood film showed many macrocytes and microcytes, and occasional poikilocytes From a case of anorexia nervosa (× 2280)



Fig 25 *General Nutritional Anemia Bone Marrow Aspiration* The erythrogranulocytic ratio was 3 1, with most cells of the erythrocytic series in the early, intermediate, and late erythroblastic stages, and with relatively few normoblasts present, thus indicating a maturation defect in this series Hypochromia was not so marked in this case, although for many months the patient's diet had been restricted largely to potato soup. A sharp rise in reticulocytes followed adequate therapy, the reticulocyte count falling as normal hemoglobin and red blood cell levels were restored (× 2280)

reticulocytosis denotes an active bone marrow up to the point of marrow exhaustion, and adequate treatment is followed by a temporary increase in reticulocytes The leukocyte count may be normal, but is usually decreased, the differential count not changing beyond a neutropenia when the total count is low. The few normoblasts present (Fig. 25). In other instances, maturation proceeds along more nearly normal lines (Fig 26).

When inanition is severe and prolonged, the so-called "starvation marrow" follows. Here the hematopoietic tissue has been depleted and partly to completely replaced by fat, which

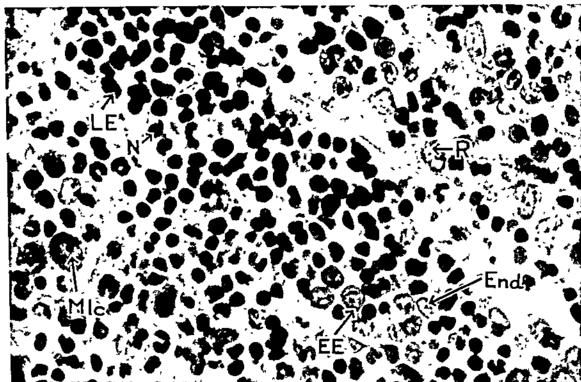


Fig 26 *General Nutritional Anemia Bone Marrow Section.* The specimen was taken from a sixteen-month-old war orphan who was emaciated and extremely pallid The hospital notes included the diagnosis of severe hypochromic anemia, but blood counts were not recorded. The child died of confluent bronchopneumonia the day after admission The bone marrow is totally cellular and the bulk of cells belong to the erythrocytic series (EE, early erythroblast, LE, late erythroblast, N, normoblast). A few myelocytes (Mlc), reticulum cells (R), and endothelial cells (End) are present (× 1000)

thrombocyte count is reduced in advanced cases

The *bone marrow* is hyperactive up to a certain point, frequently being completely filled with hematopoietic tissue. Red cell progenitors predominate, and in a good many cases there seems to be a maturation defect, since the majority of these cells are in the early, intermediate, or late erythroblastic stages with relatively

secondarily undergoes a change analogous to serous atrophy of body fat elsewhere, with or without deposition of fibrin (Figs. 27 and 28). Grossly, such marrow appears gelatinous, and its distribution may be diffuse or patchy. This gelatinous degeneration is also found in the marrow in exhaustion states from other causes (see Figs. 37, 136 and 259), such as untreated pernicious anemia or terminal nephritis.

Fig. 27. *General Nutritional Anemia Bone Marrow Section* This is the starvation marrow of prolonged inanition or chronic wasting diseases There has been fat replacement of hematopoietic tissue, followed by serous atrophy of the fat (× 2000)

Fig 28 *General Nutritional Anemia Bone Marrow Section* Starvation marrow (gelatinous degeneration) showing marked deposition of fibrin in the interstices (× 2000)

IRON-DEFICIENCY ANEMIAS

Simple Iron Deficiency

The average diet in this country usually contains assimilable iron in excess of the daily requirement. Chlorosis, the "green sickness," is virtually nonexistent today. The iron requisite for menstruating women is several times that of men, however, and for growing children still greater, so that a somewhat restricted iron intake in women and children still can give rise to a minor hypochromic anemia. Factors other than diet may create a state of iron deficiency in the patient, notably iron loss from slight but chronic hemorrhage (see chronic hemorrhagic anemia). Alimentary disturbances affecting absorption of iron, as well as the increased requirement during pregnancy, must also be considered.

The blood and bone marrow changes are essentially the same as those to be described under idiopathic hypochromic anemia in the following section, although they are generally not so severe.

The administration of *ferrous salts* is followed by a moderate reticulocytosis and a restoration of hemoglobin, red blood cells, and mean corpuscular volume to normal levels. The reticulocytes reach a peak about the end of the first week, and the count slowly falls as the red blood cell and hemoglobin curves continue to rise.

Idiopathic Hypochromic Anemia

This iron deficiency anemia is considered separately because it appears to be a clinical entity. Nearly all patients are women, the majority between the ages of twenty and fifty. A considerable percentage of these have been of poor economic status. A great many have had constitutional features similar to those of pernicious anemia, such as a fair complexion with light-colored, wide-spaced eyes, prematurely gray hair, and wide costal angle. A familial linkage with patients suffering from pernicious anemia has been described.

A multiplicity of factors seems to be involved in the pathogenesis of the condition, notably blood loss (menorrhagia), increased iron requirement (repeated pregnancies), inadequate iron consumption (poor diet, capricious appetite), faulty iron absorption (achlorhydria), and possibly incomplete utilization of iron. Usually more than one of these factors are contributory. In men, prolonged occult bleeding is probably of major importance.

Virtually all patients complain of feeling tired constantly. Many have symptoms referable to the gastro-intestinal tract, from sore tongue to constipation. The glossitis is sometimes accompanied by papillary atrophy of the tongue, but never as marked as one finds in pernicious anemia. Dysphagia is striking in some instances, specifically designated as the Plummer-Vinson syndrome; this is due in part to stricture of the upper esophagus, although symptoms are relieved by iron therapy even without demonstrable change in the roentgenograms of the esophagus. These digestive disturbances serve to establish a vicious cycle whereby food intake (and consequently iron) is further restricted.

Palpitation and cardiac arrhythmias are commonly encountered, as well as facial and ankle edema. Tingling and numbness of the extremities occur, but rarely if ever in association with positive neurologic signs of posterolateral sclerosis. Menorrhagia is an important factor in a third or more cases, often relieved as blood levels are restored to normal by iron therapy.

The pallor is gray to gray-brown, sometimes simulating the discoloration of Addison's disease; I have seen several patients who had been treated with adrenal cortical extract because of weakness and gray-brown tinge to the skin. The relation of iron to epithelium is illustrated by the dry, inelastic skin of many of these patients, the thin, dry hair, and particularly by the nails which are frequently brittle, concave, and ridged. The corners of the mouth are apt to be fissured, and the oral mucous membrane blistered. In severe cases, an apparent cardiac enlargement is due to dilatation and is associated with hemic murmurs. The spleen is often palpable, but seldom attains remarkable size; the liver is occasionally larger than normal.

Significant Laboratory Data. Examination of the peripheral blood smear (Fig. 29) affords an excellent clue as to the diagnosis. The majority

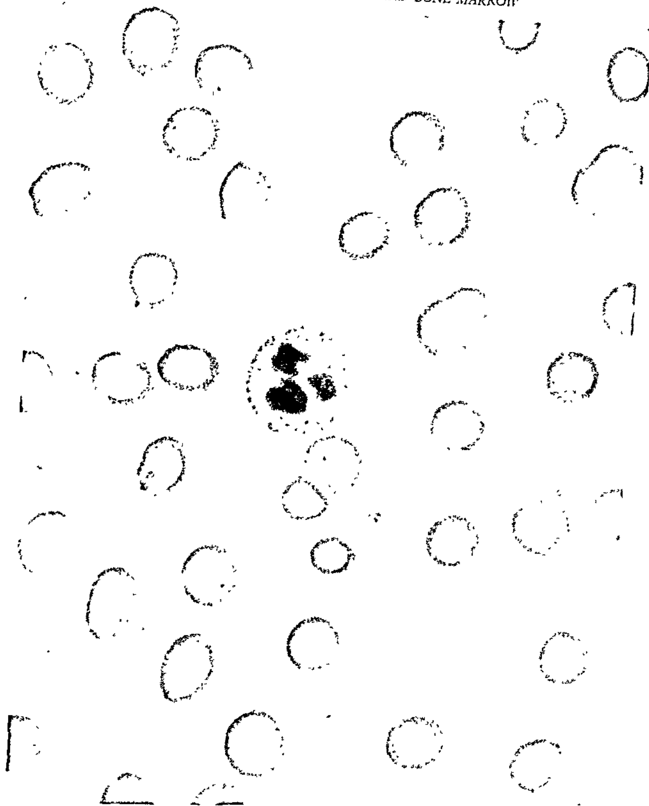


Fig 29 *Idiopathic Hypochromic Anemia Blood Film* The smear was taken from a twenty-eight-year-old white woman who complained of progressive weakness, sore mouth, menorrhagia, and vague gastro-intestinal disturbances She had [illegible] cked and sore, and her [illegible] moglobin only 4 gm, [illegible] it was not significantly altered A gastric analysis disclosed an absence of free hydrochloric acid The blood film presents the typical picture of a severe iron deficiency anemia Although a few erythrocytes appear to have their full complement of hemoglobin, in the majority, a narrow, stained rim encloses a colorless center Compared with the neutrophilic leukocyte (12 μ in diameter) in the field, the red cells are obviously smaller than normal (× 2280)

of erythrocytes display an increase in central pallor, while there is an interspersion of fully hemoglobinized cells, some of which are larger and polychromatophilic (reticulocytes). Comparison of red cell diameter with that of leukocytes shows the majority to be microcytic. A scattering of macrocytes is usually present, for this reason the estimation of average red cell diameter by halometric means is sometimes in error.

The usual laboratory findings in idiopathic hypochromic anemia are noted in the following tabulation:

TABLE 9

LABORATORY FINDINGS IN IDIOPATHIC HYPOCHROMIC ANEMIA

Finding	Result
Erythrocytes	Normal or decreased
Hemoglobin	Disproportionately low
Erythrocyte diameter	3 to 12 μ, average 6 to 7 μ
Mean corpuscular volume	Decreased (50 to 80 cu μ)
Volume index	Decreased (0 5 to 0 8)
Mean corpuscular hemoglobin concentration	Decreased (20 to 30%)
Mean corpuscular hemoglobin	Decreased (15 to 25)
Color index	Decreased (0.3 to 0 8)
Saturation index	Decreased (0 6 to 1 0)
Erythrocyte resistance (hypotonic saline solution)	Normal or increased
Reticulocytes	Normal or less
Leukocytes	Normal or less
Thrombocytes	Normal or slightly reduced
Sedimentation rate	Normal limits (corrected)
Plasma iron	Low (35 μ gm per 100 ml)
Erythrocyte porphyrin	High (1–500 μ gm per 100 ml red blood cells)
van den Bergh reaction	Negative
Serum bilirubin	Normal
Gastric analysis	
Free HCl	Decreased or absent
Total acid	Decreased
Pepsin	Decreased
Mucus	Increased
Occult blood in feces	Frequently positive

Adequate treatment with ferrous salts initiates a reticulocyte crisis similar to that of pernicious anemia with liver therapy, but not so pronounced Symptomatic response is often as dramatic as in pernicious anemia, and the hemoglobin and red cell curves usually reach normal within two or three weeks, rarely as long as six weeks. Administration of hydrochloric acid by mouth may hasten recovery, but it is not necessary.

BONE MARROW. The bone marrow is the seat of exceedingly active erythropoiesis, with maturation proceeding along normal lines and the majority of cells in the erythroblastic and normoblastic stages (Fig. 30). In severe cases, poverty of hemoglobin is apparent in the more mature nucleated red cells, normoblasts sometimes appearing as naked nuclei with a colorless halo (Fig. 31). Activity of the neutrophil precursors may be depressed and result in a moderate degree of neutropenia; eosinophils are usually normal in number or slightly increased. I have seen a moderate eosinophilia of the marrow develop during the course of treatment with iron. After normal blood levels have been restored and an adequate iron intake maintained, the bone marrow reverts to a virtually normal state.

Other Hypochromic Anemias

Many anemias other than those due to iron deficiency alone may be characterized in part or throughout their course by disproportionately low hemoglobin levels. This is often due to the fact that organic compounds of iron stored by the reticulo-endothelial system of the body are less readily utilized than inorganic (ferrous) iron absorbed from the gastro-intestinal tract. The recovery phase of pernicious anemia offers a good example of this situation, where the red cell and hemoglobin curves frequently cross, requiring administration of supplementary iron Many cases of hemolytic anemia are hypochromic for this same reason, despite a rather pronounced hemosiderosis of the tissues.

Pappenheimer and co-workers* described cases of severe hypochromic anemia in which iron-staining bodies appeared in erythrocytes after removal of an enlarged spleen (Fig. 32). The red cell inclusions resembled Heinz-Ehrlich bodies produced by acetylphenylhydrazine, except that they gave a positive Prussian-blue reaction. They are probably related to the siderocytes described by Doniach and associates † The patients failed to respond to the usual anti-anemic measures.

* Quart. J Med., *14* 75, 1945.
† J Path & Bact, *55* 23, 1943

Fig 30 *Idiopathic Hypochromic Anemia Bone Marrow Aspiration* The erythrogranulocytic ratio in this case was about 5·1, with many early erythroblasts, most cells of the erythrocytic series are in later stages of development (× 2280)

Fig 31 *Idiopathic Hypochromic Anemia Bone Marrow Section* The preponderance of red cell progenitors is less marked in this case than in the marrow illustrated in Fig 30 The disparity between the red cell count and the hemoglobin level was very pronounced (red blood cells, 4,300,000 per cu mm , hemoglobin, 3 4 gm) The cytoplasmic pallor of the late erythroblasts and normoblasts is unusually evident in this section (× 1000)

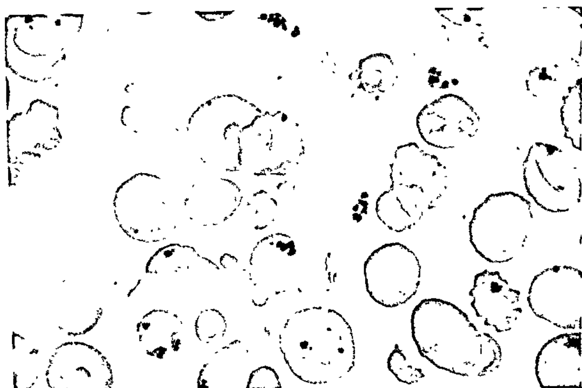


Fig 32 *Hypochromic Anemia with Siderous Bodies. Blood.* An extreme degree of hypochromia is noted in the erythrocytes containing small, dark bodies which react positively for iron Anisocytosis and poikilocytosis are pronounced (× 2100) (Slide, courtesy of Dr Alwin M Pappenheimer)

DEFICIENCY OF THE HEMATOPOIETIC PRINCIPLE

Pernicious Anemia

This severe macrocytic anemia, fatal if untreated, occurs in middle and late life, mostly in the white race There is a familial predisposition to the development of the disease Constitutional factors are also noted in many patients; such as prematurely gray or white hair of silky texture, light-colored eyes widely spaced, and a broad costal angle These features are sometimes noted in patients with idiopathic hypochromic anemia, and this disease has been observed in families in which pernicious anemia has also occurred. This linkage is probably based on the gastric dysfunction common to the two types of anemia, since achlorhydria occurs more frequently in these families than in the general population

The present concept regarding the pathogenesis of pernicious anemia is patterned on Castle's theory that a *hematopoietic principle* is required for the orderly maturation of red blood cell progenitors in the bone marrow. This principle is elaborated through the interaction of an *intrinsic factor* secreted by the stomach and duodenum, and an *extrinsic factor* in the diet. It is stored chiefly in the liver and is withdrawn as required for blood formation. Persons suffering from pernicious anemia apparently lack the intrinsic factor, as a result of atrophy of the gastric glands. There is evidence that vitamin B_{12} may be the hematopoietic principle, and that the intrinsic factor governs its absorption from the intestine (see p. 19)

Clinical features of the disease may be referable to the anemia per se, to the gastro-intestinal disturbances, to the posterolateral degeneration of the spinal cord, or any combination thereof In virtually no other condition does the patient remain ambulatory with such low red cell counts as in pernicious anemia, a point of some diagnostic value. Gastro-intestinal or neurologic symptoms occasionally antedate the anemia, however, and spontaneous remissions may prove confusing.

The most common complaints are weakness, dizziness, sore tongue, diarrhea, and numbness and tingling of the extremities. Some patients lose weight, while others maintain a good nutritional status. The skin and mucous membranes are pallid, and a faint icteric tinge is occasionally seen. Repeated attacks of glossitis generally leave a smooth tongue, although the tongue may be smooth without ever having been sore. Patients with spinal cord and peripheral nerve degeneration show loss of reflexes, and impaired sense of position and vibration.

Significant Laboratory Data. Blood. On examination of the stained blood film, one is impressed by the large size, rich coloration, and frequently bizarre shape of the erythrocytes (Fig. 35). This deviation from the circular form creates difficulty in the preparation of a Price-Jones' curve (Fig. 33), but with careful meas-

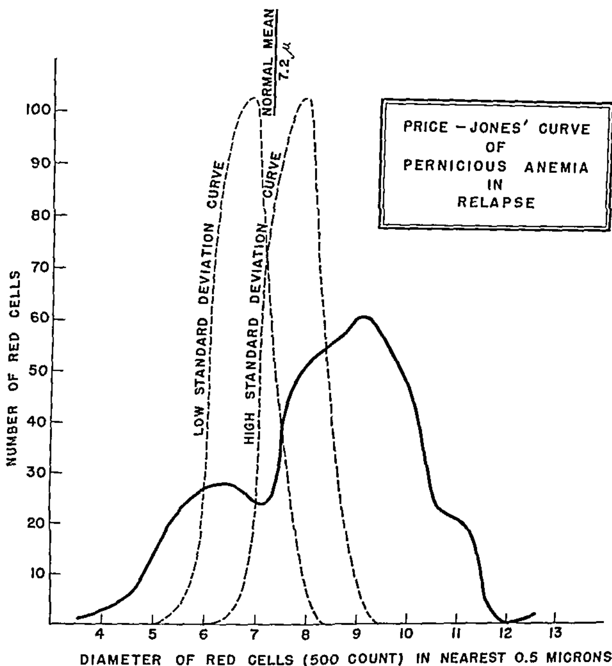


Fig. 33. *Price-Jones' Curve of Pernicious Anemia in Relapse.* The broad base of the curve and the low peak lying to the right of normal are the characteristic features, indicating predominantly large red blood cells in association with many microcytes.

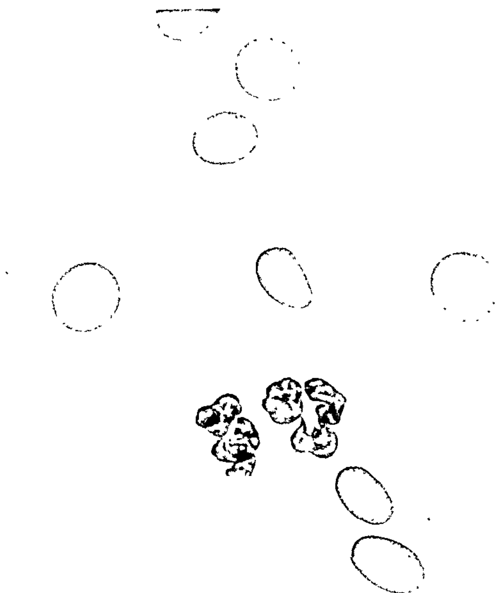


Fig 34 *Pernicious Anemia Blood* Two "macropolycytes" 15 and 18 μ in diameter, with complex nuclei are seen The erythrocytes here are generally large and not particularly distorted, so that a casual examination of the blood smear might not disclose these several features of diagnostic significance (× 2100)

urement of the cell diameter, the curve also discloses a surprising number of very small cells. The peak of the curve falls considerably to the right of the normal, however, accounting for an increase in the mean corpuscular volume and color index, the latter despite a normal or slightly decreased mean corpuscular hemoglobin concentration. Nucleated red cells appear inconstantly and vary from partly hemoglobinized megaloblasts to normoblasts which are occasionally very large, sometimes with multilobate ("cloverleaf") nuclei. A reticulocyte crisis following liver therapy may be associated with a shower of nucleated forms, usually normoblasts, in contradistinction to the flood of megaloblasts and erythroblasts which occasionally mark a serious relapse. Blood films taken during the early days of treatment show many red cells with diffuse and punctate basophilia (reticulocytes). Cabot rings and Howell-Jolly bodies are commonly seen.

Leukopenia, a rather uniform finding, is due to a reduction in neutrophils which display a "shift to the right," characterized by large, multilobate "macropolycytes" (Fig. 34). Some authors regard this as due to suppression and delayed delivery of neutrophils from overactivity of erythropoietic tissue, it is more likely that the hematopoietic principle exerts an influence on this developmental series as well as on the erythrocytic series. Immature cells of the granulocytic series occasionally appear in the peripheral blood Eosinophilic leukocytosis has been noted in some cases.

The thrombocyte count is often reduced, roughly in proportion to the erythrocyte level, related to an actual depletion of megakaryocytes in the bone marrow. Here, too, this reduction is either a "crowding out," or due perhaps to lack of the hematopoietic principle Clot retraction is relatively slowed, and the bleeding time occasionally increased

Laboratory studies of value in the diagnosis of pernicious anemia are listed below, and the deviation from normal indicated. Those marked by an asterisk are absolutely essential for diagnosis

TABLE 10

LABORATORY FINDINGS IN PERNICIOUS ANEMIA

Test	Finding
*Erythrocytes	Decreased (as low as 500 000 per cu mm)
*Hemoglobin	Disproportionately high
Erythrocyte diameter	4 to 12 μ, average 8 to 9 μ
Mean corpuscular volume	Increased (100 to 150 cu μ)
*Volume index	Increased (1 2 to 1 6)
Mean corpuscular hemoglobin concentration	Normal or slightly decreased (28 to 38%)
Mean corpuscular hemoglobin	Proportionate to red blood cell size
*Color index	Increased (1 2 to 1 8)
Saturation index	Normal or decreased (1 to 0 8)
Red blood cell resistance:	
(hypotonic saline solution)	Normal or slightly increased
(saponin)	Markedly decreased
*Reticulocytes	Increased early, decreased later
*Leukocytes	Decreased (neutropenia)
*Thrombocytes	Decreased
*Sedimentation rate	Normal, if corrected
van den Bergh reaction	Indirect
Serum bilirubin	Increased
*Gastric analysis (histamine)	
Free HCl	Absent
Total acid	Low
Pepsin	Low or absent
Mucus	Decreased or absent
Serum uric acid	Increase with remission

BONE MARROW FINDINGS Since the advent of liver therapy, one rarely has the opportunity to perform the autopsy on a patient with pernicious anemia who has died in a relapse. On such occasion, however, the marrow of virtually all bones, flat and long alike, is found to be dark red, glistening, jelly-like tissue, frequently spotted with yellowish patches; that is, areas of relative aplasia (see frontispiece). A more widespread terminal aplasia is seldom seen (Fig 37). The gross appearance of the marrow in adequately treated persons who have died of some unrelated condition does not differ from the usual.

The cytologic features of the bone marrow during relapse are shown in an aspiration biopsy of the sternum (Fig. 38). Megaloblasts take the foreground, most have deeply basophilic cytoplasm, and some have already acquired hemoglobin. The erythroblasts and normoblasts are less conspicuous Many early

Fig 35. *Pernicious Anemia. Blood* Wide range in red cell diameter and the poikilocytosis are more usual findings in the peripheral blood than the uniformity seen in Fig 34 (× 2100)

Fig 36 *Pernicious Anemia. Blood* Blood smear stained with brilliant cresyl blue taken during a reticulocyte crisis nine days after treatment with liver extract was begun (× 1500).

erythroblasts that would normally have blue cytoplasm show a distinct pink tinge, and the precocious acquisition of hemoglobin is still more evident in the intermediate (normally polychromatophilic) erythroblasts. The marked variation in size and shape of erythroblasts is striking, some being huge. Mitotic figures are frequently encountered in this series, mostly in megaloblasts, and may be atypical.

There is a relative paucity of cells of the granulocytic series, although eosinophils are usually conspicuous. Abnormally large neutrophilic myelocytes, metamyelocytes, and segmented forms are found with fair regularity. Megakaryocytes are either decreased, normal, or increased in numbers, some show agranular, deeply basophilic cytoplasm.

The true pattern of the marrow is disclosed only by sections of the fixed tissue, despite the shrinkage due to fixation (Fig. 39). In the average case, virtually all fat has been replaced by hematopoietic tissue. The same megaloblastosis and imperfect maturation in the erythrocytic series are evident, along with certain other features. The megaloblasts tend to form clumps, and even syncytial masses where they appear to be but little removed from undifferentiated reticulum cells. The disproportion between early and late forms of the erythrocytic series and between progenitors of red cells and cells of the granulocytic series is more clearly seen than in smears.

Changes During Remission. The remission induced by administration of potent liver extract (or by vitamin B_{12}, or folic acid) is characterized by changes in the bone marrow that are far more dramatic than those in the blood. This can be seen in sternal aspirates taken six hours after the first liver injection, and by the end of the first day the swing toward a predominantly erythroblastic type of cell is well established (Fig. 40). By the fourth day, fewer megaloblasts are left, and normoblasts are found in profusion (Fig. 41). A biopsy taken on the tenth day (Fig. 42) shows normoblasts in preponderance and not many megaloblasts. After normal blood levels have been reached, the activity of the marrow be-

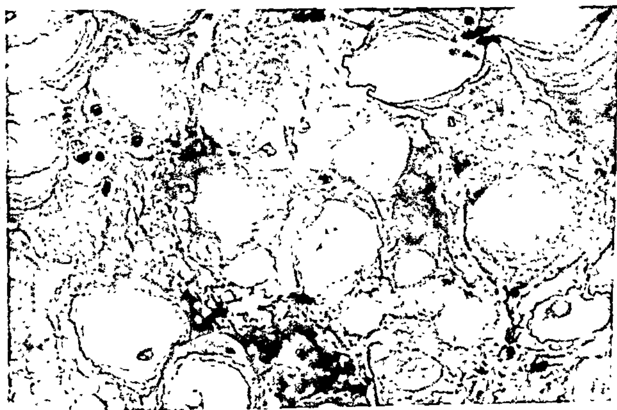
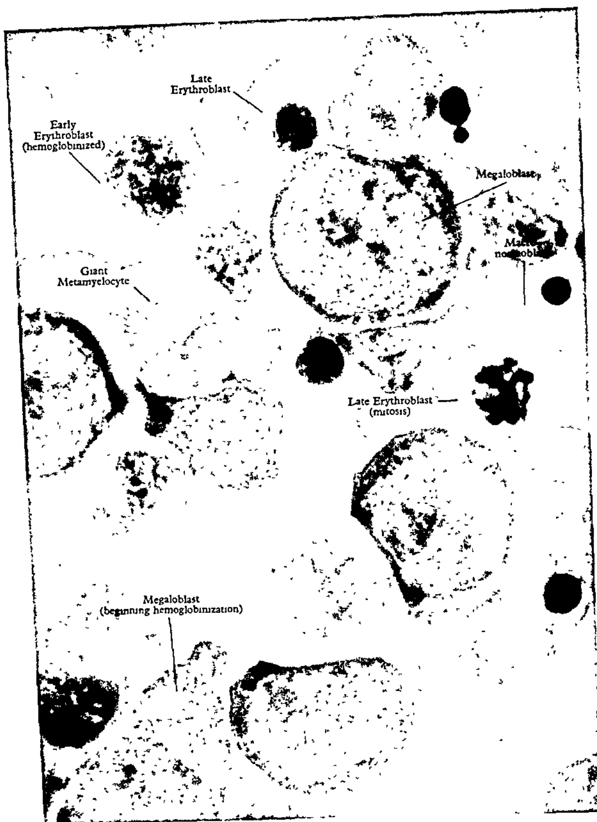


Fig 37 *Pernicious Anemia Bone Marrow Section, Terminal Exhaustion State.* From a patient who died before the advent of liver therapy. Hematopoietic tissue in all bones studied was largely replaced by fat which had undergone serous atrophy (see starvation marrow, Figs 27 and 28) (× 500)


Late
Erythroblast
Early
Erythroblast
(hemoglobinized)
Megaloblast
Giant
Metamyelocyte
Late Erythroblast
(mitosis)
Megaloblast
(beginning hemoglobinization)

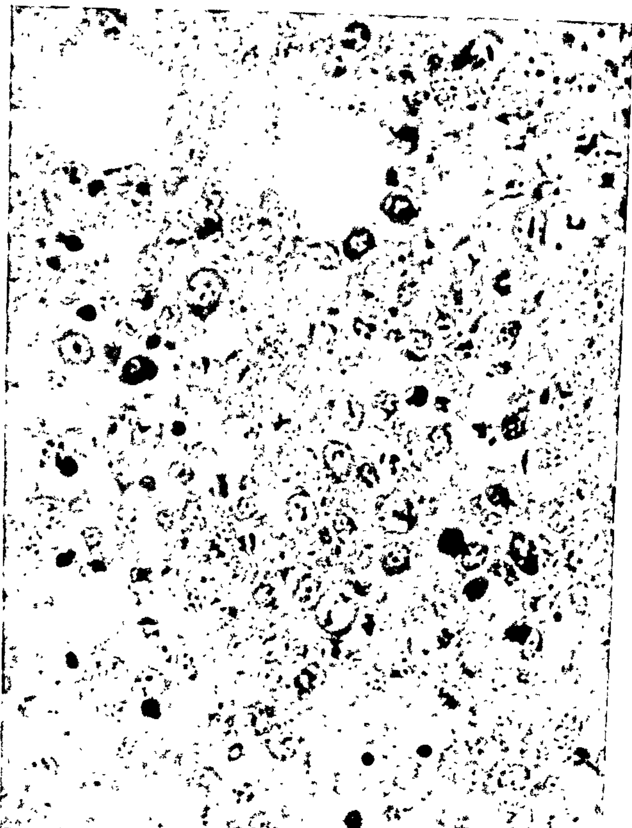
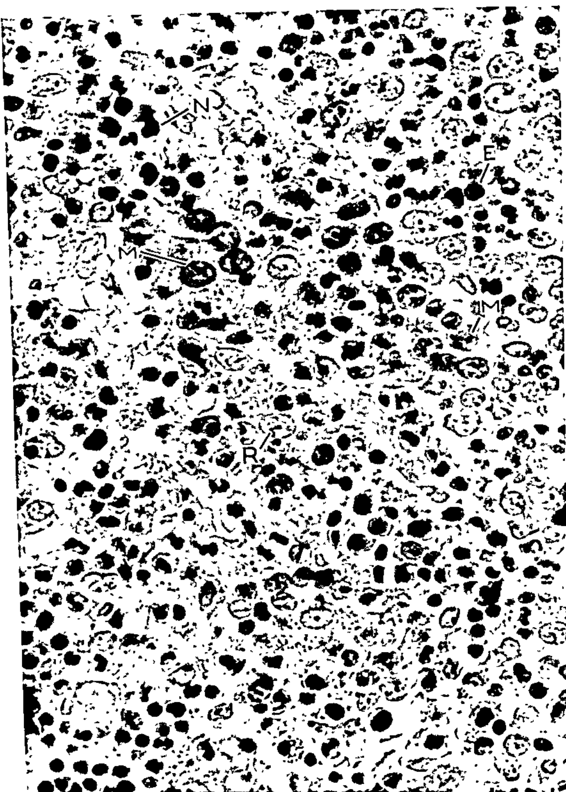


Fig 39 *Pernicious Anemia Bone Marrow Section During Relapse* The bulk of the cells are megaloblasts having a large vesicular nucleus, with nucleoli or coarse chromatin bars and a narrow rim of basophilic cytoplasm, very few erythroblasts and normoblasts (the cells with dark nuclei) are present No megakaryocytes appear in the field pictured, and there were scarcely any in the aspirated fragments of the marrow Cells of the granulocytic series were unusually sparse in this case (× 1000)


N
E
M
M
R

[illegible]

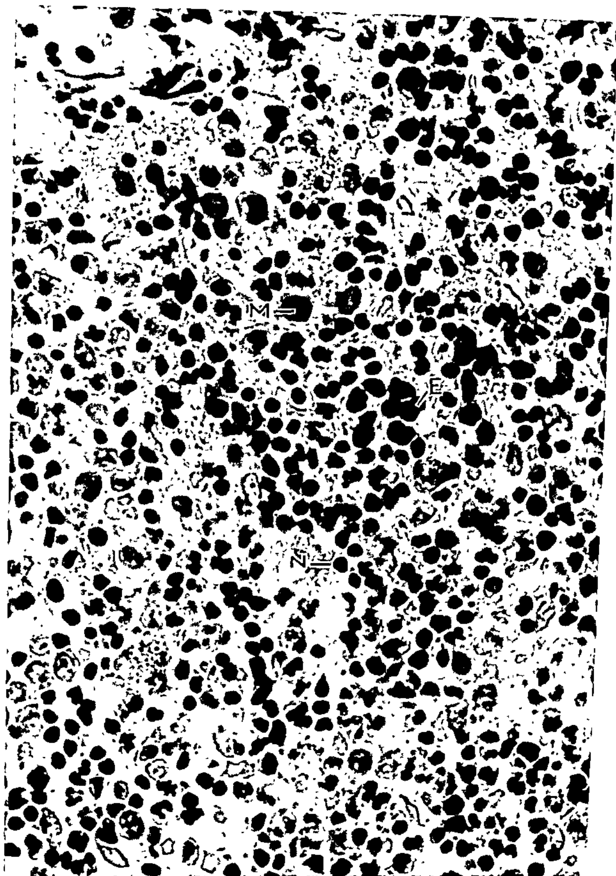
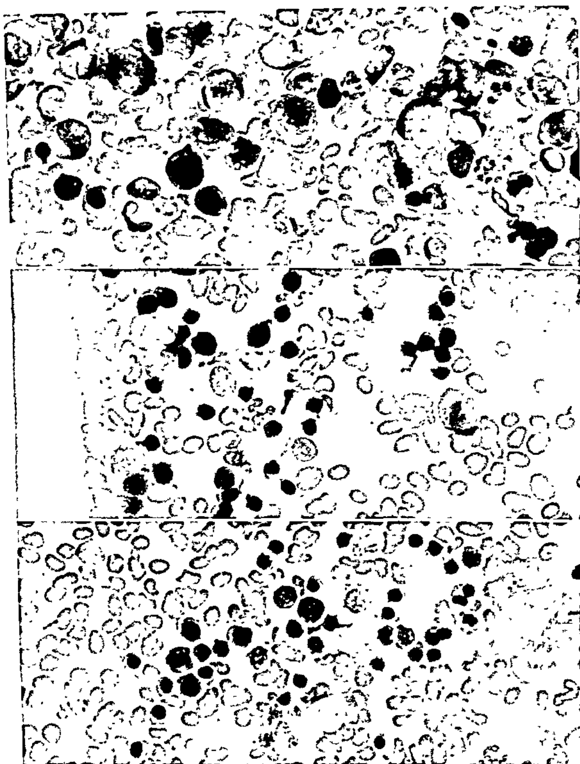


Fig 41 *Pernicious Anemia Bone Marrow Section in Early Remission.* Four days after institution of liver therapy, megaloblasts (M) are far in the background, while erythroblasts (E) and especially normoblasts (N) have increased tenfold or more (× 1000).



an almost completely normoblastic marrow Even inadequate liver therapy may initiate this change, and obliterate diagnostic features of the marrow biopsy (all × 650)

comes progressively less, and fat is restored in its usual proportion for the age of the patient Even so, an occasional megaloblast may be found in the marrow of persons whose blood picture has been normal for a long time.

A difference of opinion exists regarding the *manner in which the therapeutic agent effects a* remission Some investigators believe the abnormal megaloblasts are promptly switched to a normal maturation sequence (Table 11); others believe they continue their abnormal course and are gradually replaced by erythropoietic tissue which develops in a natural fashion. *The speed with which the changes in the* marrow come about would support the former theory.

Treatment During the first two weeks after the institution of specific therapy, the red cell, hemoglobin, and reticulocyte levels must be followed closely, preferably daily, then once each week until remission is completely established The curves in one of our cases are shown in Fig. 43, the reticulocyte peak is usually reached later (seventh to tenth day) and may be much higher. Purified *liver extract* (15 units per ml.) is the accepted therapeutic agent, 1 ml. being given intramuscularly each day for the first two weeks, and at weekly intervals until the blood picture is quite normal. Thereafter, 15 units every four to six weeks will keep the patient in good health. It is sometimes necessary to administer *ferrous salts* during the period of regeneration if the hemoglobin curve lags. *Folic acid* (pteroyl glutamic acid) should be used only in conjunction with purified liver extract; alone, it will effect as good a hematopoietic response, but has no value with respect to changes in the nervous system Indeed, posterolateral sclerosis has developed in a number of patients whose blood levels had been restored to normal and maintained there by folic acid Dameshek* recommends the use of folic acid to fill the gap be-

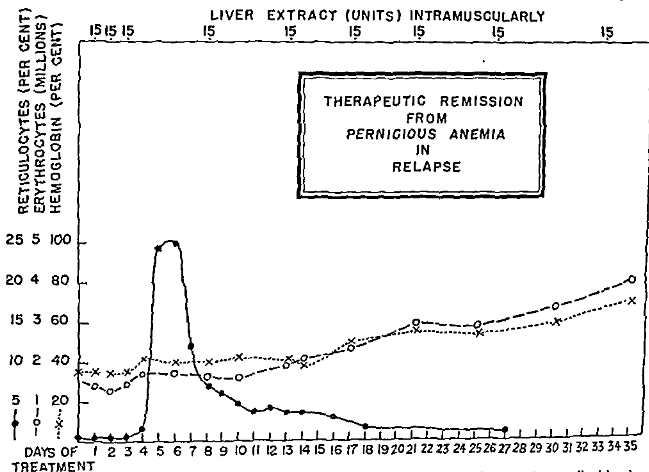


Fig 43 *Pernicious Anemia Blood Levels During Therapeutic Remission* The reticulocyte peak is usually delayed several days beyond that shown in this case, and often reaches heights of 40 to 60 per cent, sometimes more. Red blood cell regeneration may so outspeed hemoglobin that administration of iron becomes necessary.

* Blood *3* 699, 1948.

tween injections of liver extract. For economic reasons, we do not give folic acid to our Hematology Clinic patients, and they seem to get along quite as well without it.

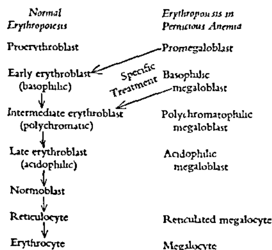
Vitamin B_{12} has not yet proved to be any better than purified liver extract in the treatment of pernicious anemia. Its value thus far has been as a tool in better understanding the mechanism of the disease.

Patients who have been inadequately treated with liver or who have had a relapse after discontinuing treatment do not show such a spectacular reticulocyte crisis as does the new patient; the peak may not reach more than 5 to 10 per cent, and the rise in red cells and hemoglobin is likely to be more gradual.

I cannot help interjecting a comment on the use of compound hematinics (capsules of liver extract, desiccated hog stomach, multiple vitamins, folic acid, iron, copper, and whatnot) in the treatment of pernicious anemia (or any other anemia). Such treatment is inadequate to bring about a complete remission, and it destroys the specificity of the blood and bone marrow pictures to such an extent that accurate diagnosis is virtually impossible. This is a lazy, unscientific practice that benefits only the manufacturer, and is to be condemned.

TABLE 11

EFFECT OF SPECIFIC TREATMENT ON ERYTHROPOIESIS IN PERNICIOUS ANEMIA*



Normal Erythropoiesis	*Erythropoiesis in Pernicious Anemia*
Proerythroblast	Promegaloblast
Early erythroblast (basophilic)	Basophilic megaloblast
Intermediate erythroblast (polychromatic)	Polychromatophilic megaloblast
Late erythroblast (acidophilic)	Acidophilic megaloblast
Normoblast	
Reticulocyte	Reticulated megalocyte
Erythrocyte	Megalocyte

* The speed with which marrow transformation comes about is best explained on the basis of the altered maturation course indicated above.

Pernicious Anemia of Pregnancy

Although rare, this disease seems to be an entity. It usually appears during the third trimester and is frequently associated with fever and symptoms referable to the gastro-intestinal tract; purpuric phenomena have been described. The *blood picture* is similar in many respects to that of primary pernicious anemia (p. 50), but variations in size and shape of erythrocytes are not usually so marked, and neutrophilic leukocytosis is apt to be present. Reticulocytes may be normal or increased in number. Evidences of abnormal hemolysis have been noted in some cases, while others have shown no increase in serum bilirubin or urobilinogen excretion. The majority of patients have had normal gastric juice. The *bone marrow* has been described as megaloblastic, resembling pernicious anemia in relapse. In the two cases that I have studied, megaloblasts were present, but there was an associated normoblastic component, and in each instance the picture more closely resembled that of pernicious anemia a day or two after liver therapy had been instituted (Fig. 40). This disease also differs from primary pernicious anemia in its response to parenteral administration of concentrated liver extract, being rather refractory, while folic acid, crude liver extract, or autolyzed yeast by mouth usually induces a prompt remission. A final difference is the spontaneous cure that follows termination of the pregnancy.

Macrocytic Anemia of Infancy (Infantile Form of Pernicious Anemia)

A macrocytic anemia (Fig. 44) occurring in infants has been described, associated with neutropenia and thrombocytopenia. Achlorhydria (refractory to histamine) has been demonstrated in some cases. The bone marrow is megaloblastic (Fig. 45) and contains giant metamyelocytes. It is thought to be due to a congenital absence of the intrinsic factor and has been corrected by the administration of liver extract or folic acid in much the same fashion as pernicious anemia. Some patients have made a complete and permanent recovery, while others have required con-

tinuous liver therapy to maintain normal hematopoiesis.*

Sprue and Allied Conditions

Tropical sprue, idiopathic steatorrhea (nontropical sprue), celiac disease (nontropical sprue in infancy and early childhood), nutritional macrocytic anemia, and macrocytic anemias resulting from gastro-intestinal fistulas will be considered as a group, because they have a number of features in common The anemia in each, when it occurs, is apparently due to deficiency or faulty absorption of the hematopoietic principle. In the first three conditions, the causative factors underlying the disease are not well understood, but dietary (vitamin?) deficiencies unquestionably play a part

Tropical sprue may occur at any age, most commonly between twenty and forty, and is especially apt to affect white adults from temperate zones who are living or have lived in the tropics. *Idiopathic steatorrhea* is a disease of puberty or beyond, while celiac disease is essentially the same condition developing between the ages of six months and three years. The prodrome of ill-defined gastro-intestinal disturbances may extend for many years, or the onset may be relatively sudden. There is a wide range in the severity of symptoms, and degree of anemia in different persons Sore and *eventually smooth tongue, diarrhea with foul,* bulky stools of high fat content, anemia which is usually macrocytic, pallor, weakness, and loss of weight should indicate the *sprue* disease family. Roentgenographic study of the intestinal tract discloses segmental dilatation, mucosal distortion, and defective motility which are regarded as characteristic Calcium deficiency is frequently noted in idiopathic steator-

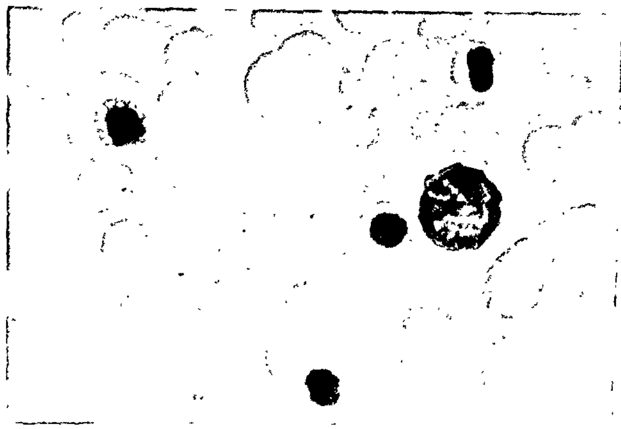


Fig 44 *Infantii* moribund state. 1,100,000 per cu hypersegmented neutrophils, thrombocytes, 68,000 per cu mm (indirect) A fair number of red cells appeared macrocytic A blood film from an earlier count (figures not obtained) disclosed many fewer nucleated red cells, and a greater degree of macrocytosis There was a history of increasing pallor, anorexia, and failure to gain weight normally during the previous six months (· 2100)

* Am J Dis Child, 75 143, 1948

TABLE 12

COMPARATIVE FINDINGS IN THE "PERNICIOUS ANEMIA FAMILY"

	Pernicious Anemia	*Nutritional Macrocytic Anemia*	*Sprue*	*Idiopathic Steatorrhea*	*Celiac Disease*
Psychoses	+	++			
Neurologic involvement	++	+	+		
Absence of intrinsic factor	+++	+	+		
Achlorhydria	+++	++	+	+	
Macrocytic anemia	+++	+++	++	+	
Glossitis	++	+++	+++	+	
Esophagitis, proctitis, vaginitis		+++	+++	+	
Diarrhea	+	++	+++	++	++
Weight loss	+	++	+++	+++	+++
Skin changes (pigmentation, etc)	+	+++	++	++	++
Anasarca	+	+	+	+	++
Steatorrhea		+	+++	+++	+++
Abdominal distention and dilatation of colon		+	+++	+++	+++
Flat blood sugar curve			++	+++	+++
Low Ca^{++} and tetany			+	+++	+++
Hypochromic anemia		++	+	++	+++
Onset in childhood.			+	++	+++
Bone deformities				+++	+++
Infantilism				+++	+++
Erythroblastic anemia				+	+

(From Wintrobe, M M Clinical Hematology Lea & Febiger)

rhea and celiac disease, owing to a combination of calcium with excess fat in the stool, so that tetany and bone changes may occur

Significant Laboratory Data. Table 12 (Wintrobe) presents the more important findings in the sprue group of diseases, and indicates the relative frequency of each in comparison with pernicious anemia

Idiopathic steatorrhea and celiac disease can be differentiated from the steatorrhea of pancreatic dysfunction, since in the first two the fat in the stool takes the form of fatty acid needles, while in pancreatic deficiency, it is neutral fat

BLOOD. Examination of the blood in tropical sprue usually discloses a macrocytic anemia which is often normochromic or even hypochromic, with somewhat less marked variation in size and shape of erythrocytes than one finds in the relapse phase of pernicious anemia (Fig 46) The anemia may be hypochromic and microcytic in some cases, and in others there is no anemia at all The resistance of red cells to hypotonic saline solutions is normal Neutropenia is commonly encountered, and "macropolycytes" (Figs. 46 and 47) and giant metamyelocytes similar to those of pernicious anemia appear Thrombocytes are often reduced in number. The anemia of idiopathic steatorrhea and celiac disease shows the same variation, but is more frequently hypochromic and microcytic, and is sometimes associated with showers of nucleated red cells.

BONE MARROW Patients with severe macrocytic anemia show bone marrow that is virtually indistinguishable from that of pernicious anemia in relapse (compare Figs. 49 and 38), with megaloblasts predominating and maturation in the erythrocytic series quite as imperfect It is more usual, however, to find a somewhat larger proportion of erythroblasts and normoblasts (Fig. 50 contrasted with Fig. 39), and not so marked a reversal in the erythrogranulocytic ratio When the anemia is normocytic or microcytic, and normochromic or hypochromic, the bone marrow hyperplasia is marked by an excess of erythroblasts and normoblasts, resembling in large measure the marrow pictured in Figs 30 and 31

Treatment. The response in the blood and bone marrow to treatment is not so dramatic as one sees in pernicious anemia, but the end result in most cases is quite as satisfactory. *Purified liver extract* alone, administered intramuscularly and unsupplemented by dietary adjustment, will often serve to bring about a



[illegible] Marrow Section (same patient as Fig. 44) The majority of cells [illegible]

Fig 46 *Tropical Sprue. Blood* This patient had a severe macrocytic anemia with a volume index of 1 3 and color index of 1 1 There is a moderate degree of anisocytosis and poikilocytosis A "macropolycyte" is present in the field (× 2100)

Fig 47 *Tropical Sprue Blood* The anemia in this case was relatively mild. Erythrocytes are normal in size and shape, and slightly hypochromic Two "macropolycytes" are shown (× 2280).

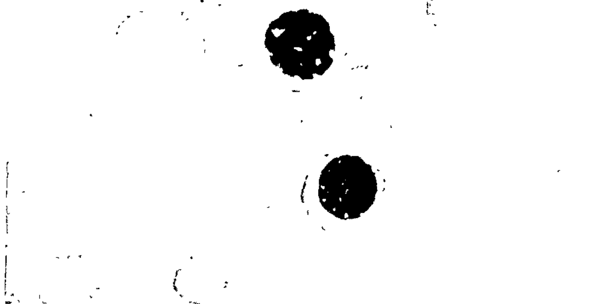


Fig. 48 *Idiopathic Steatorrhea with Erythroblastic Anemia. Blood* The anemia in this instance was microcytic and hypochromic, but with considerable numbers of nucleated red cells appearing inconstantly in the peripheral blood (× 2100)

remission. *Folic acid* is generally much more effective than liver extract in these conditions, and may restore normal blood levels in patients where liver therapy has failed. One should regulate the diet to insure a *high protein*, and *low fat* and *carbohydrate intake*. In established cases of sprue, it is usually necessary to continue therapy in some form for life, in accordance with the needs of the patient. *Surgical correction* of gastro-intestinal fistulas usually effects a cure, although remissions can be induced by liver therapy.

Gastric and Intestinal Fistulas and Strictures

Gastro-enteric, gastrocolic, entero-enteric or enterocolic fistulas, following surgical anastomosis or developing from some other cause, occasionally produce a clinical picture suggestive of sprue in some cases, and of pernicious anemia in others. The same may be said for single or multiple strictures in the intestine, especially in the ileum *

The anemia is macrocytic and normochromic or hypochromic. The bone marrow shows some degree of megaloblastosis (Fig. 51). I have seen several patients whose nutritional status was extremely bad, and whose marrow was depleted of hematopoietic tissue, along with serous atrophy of fat ("starvation marrow") (Fig. 52).

The anemia can be relieved by administering folic acid or liver extract, or by surgical correction of the lesion.

Liver Disease

Deficient storage of the hematopoietic principle is regarded as one cause of the macrocytic anemia observed in certain patients with widespread liver disease of long duration. Inadequate diet, as is found in alcoholic persons, and perhaps faulty absorption from the gastro-intestinal tract may also be causative factors. The blood picture can simulate that of pernicious anemia rather closely, but the anemia is rarely if ever as severe. Parenteral liver therapy will sometimes induce a reticulocytosis and a rise in the red cell level. The clinical features usually point directly to liver damage and are at suffi-

* See Barker and Hummel Bull Johns Hopkins Hosp, 64 215, 1939

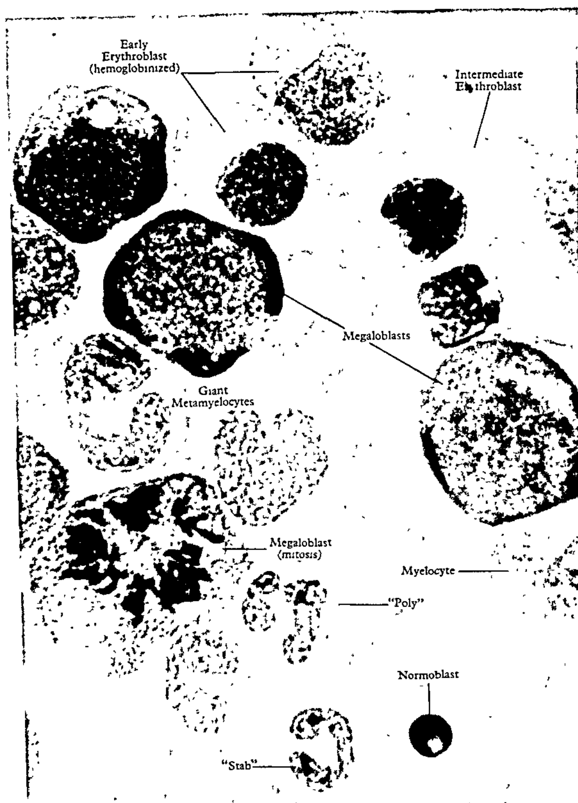


Fig 49 *Tropical Sprue Bone Marrow Aspiration* The patient was suffering from a profound macrocytic anemia This marrow is virtually indistinguishable from that of pernicious anemia in relapse (compare with Fig 38) (× 2280)

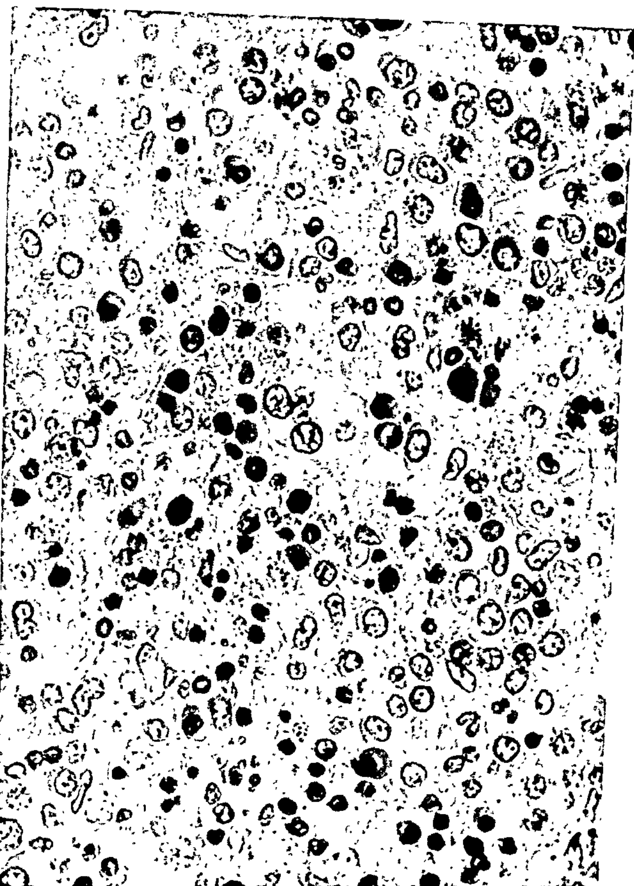


Fig 50 *Tropical Sprue Bone Marrow Section* From a fatal, untreated case of macrocytic anemia All fat is displaced by hematopoietic tissue Megaloblastosis is evident, but there is a somewhat larger proportion of erythroblasts and normoblasts present (cells with darker nuclei) than is generally found in pernicious anemia (compare with Fig 39) The complement of cells of the granulocytic series, not so evident in the black and white photomicrograph, is also greater (× 1000) (Tissue, courtesy of Dr Enrique Koppisch, School of Tropical Medicine, San Juan, Puerto Rico)

Fig 51. *Gastrocolic Fistula Bone Marrow Section* The patient was a white man of twenty-seven who had suffered a gunshot wound of the abdomen and survived the primary repair of stomach and bowel perforations, but later developed

MY

N

Fig 52 *Gastrocolic Fistula Bone Marrow Section* This elderly man developed a gastrocolic fistula near the site of a

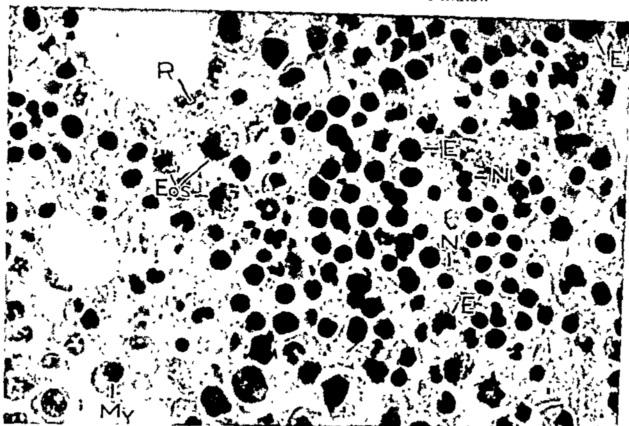


Fig 53 *Cirrhosis of Liver with Macrocytic Anemia Bone Marrow Section* Although the patient was an elderly man, very little fat remains in the sternal marrow. The bulk of the cells belong to the erythrocytic series, the majority in the erythroblastic stage (E); those marked E' are in mitosis Normoblasts (N) are richly distributed. There is a relative paucity of neutrophilic myelocytes (My), but eosinophils (Eos) are conspicuous Megakaryocytes are sparsely distributed, none appearing in this field (× 1000)

cient variance from pernicious anemia that confusion is not likely to arise in the differential diagnosis

The bone marrow is hyperactive and shows a reversal of the erythrogranulocytic ratio (Fig. 53). A scattering of megaloblasts may be present, but erythroblasts (many of them early forms) and normoblasts predominate Cells of the granulocytic series are normal except for an occasional case showing eosinophilia of the marrow. Megakaryocytes are often reduced in numbers but show no qualitative change, the hemorrhagic manifestations in some of these cases are due more to hypoprothrombinemia than to a deficiency of thrombocytes

Achrestic Anemia

Defective utilization of the hematopoietic principle is postulated to explain the anemia in this rare condition, which is said to be virtually indistinguishable from pernicious anemia. Differential points are the presence of free hydrochloric acid in the gastric secretion in all cases, and the failure to respond satisfactorily to adequate liver therapy. The hematopoietic principle has been extracted from the livers of several fatal cases The bone marrow has been described as megaloblastic. I have not had the opportunity of studying such cases, and some doubt has been expressed regarding the existence of achrestic anemia as a disease entity.

VITAMIN DEFICIENCIES

Experimental studies have amply demonstrated the importance of certain vitamins in normal hematopoiesis, notably the B complex (especially folic acid and B_{12}). *It is not so easy to* prove specific vitamin deficiencies as the sole causative factor in human cases of anemia, as other substances necessary for blood formation are usually lacking in the diet of these patients. Inadequacy of vitamins A and D, and probably C, has no bearing on the development of anemia; the anemia sometimes seen in scurvy is usually hypochromic, and in all likeli-

hood is due to iron deficiency, hemorrhage, or both.

The relationship of the B group of vitamins to anemia is shown by the hematopoietic response to administration of autolyzed yeast, folic acid, or crude liver extract in a variety of macrocytic anemias with megaloblastic type of blood formation. This would lead one to consider a connection between the B complex and the extrinsic factor. The curative effect of folic acid in pernicious anemia as regards the blood is virtually equal to that of purified liver extract, indicating that folic acid is akin to the hematopoietic principle; its failure to benefit the neurologic manifestations of this disease as does liver shows that the two substances are not identical. Most recently the isolation of vitamin B_{12} has virtually filled this gap (see p. 19 and p. 47).

The clinical and hematologic characters of B-complex deficiency have already been mentioned under nutritional macrocytic anemias.

ENDOCRINE IMBALANCE

Thyroid Dysfunction

Patients with *hyperthyroidism* are seldom anemic to an appreciable degree, although there may be a minor decrease in hemoglobin levels. A few cases of hemolytic anemia in association with hyperthyroidism have been described. The bone marrow, however, is frequently hyperplastic (Fig. 54) with cells of the granulocytic series increased. Leukocytosis is not usual, despite this marrow hyperplasia, and leukopenia with relative lymphocytosis is not rare.

Hypothyroidism is responsible for anemia in more than 50 per cent of patients, especially in those with achlorhydria. The anemia is usually mild and normocytic in type, but it may be macrocytic and is occasionally severe, resembling pernicious anemia. Bone marrow study will rule out pernicious anemia, as the marrow in myxedema is hypoplastic (Fig 55), especially with regard to the red cell progenitors.

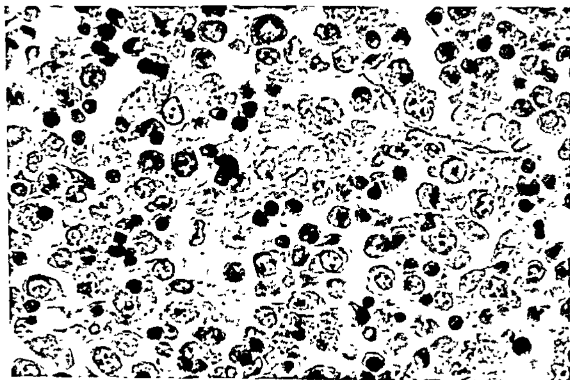


Fig 54 *Hyperthyroidism Bone Marrow Section* In this case, the marrow was unusually hyperplastic, virtually all fat having been replaced by hematopoietic tissue The vast majority of cells belong to the granulocytic series, the small cells with dark nuclei being red cell progenitors Despite the myelocytic hyperplasia, the peripheral blood did not show a leukocytosis (× 1000).

Pituitary Cachexia (Simmonds' Disease)

A moderate degree of normocytic, usually hypochromic anemia is observed in this condition. The anemia is thought by some to be due to the general malnutrition of these patients, but there may be a relation between the pituitary gland and blood formation, as anemia has followed hypophysectomy in experimental animals. In the few cases that I have examined, the bone marrow has been moderately hypoplastic.

Adrenal Cortical Insufficiency

Anemia, usually normocytic and hypochromic, is a frequent finding in Addison's disease, and is somewhat more marked in the cases due to tuberculosis than in the primary cytotoxic variety. The differential leukocyte count often shows a relative lymphocytosis and occasionally an actual increase in number of circulating lymphocytes. The anemia is difficult to explain, because the bone marrow is essentially normal.

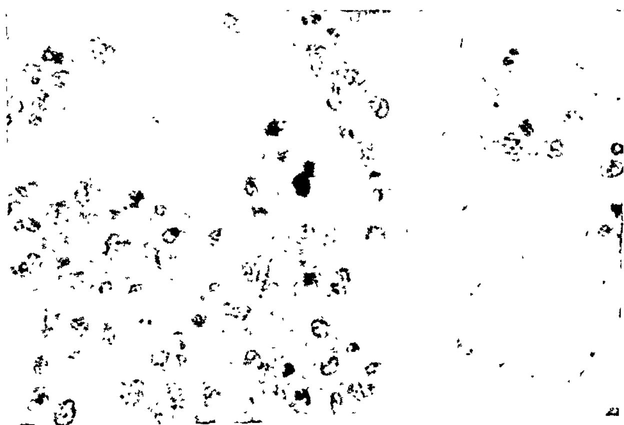


Fig 55 *Hypothyroidism Bone Marrow Section.* The section is through vertebral marrow of an elderly white woman who was admitted to the hospital in a moribund state Myxedema had not been recognized by her attending physician who had been treating her severe macrocytic anemia for several months with liver extract The marrow is largely fatty and the majority of cells are myelocytes (× 1000)

VI

APLASTIC AND HYPOPLASTIC ANEMIAS
(Panhematocytopenia)

The conditions to be considered in this section are characterized by anemia, leukopenia, and thrombocytopenia due to actual cellular depletion of the bone marrow with consequent lack of blood formation. Anemias caused by displacement of the marrow (myelophthisic anemias) and by failure of normally cellular or hyperplastic marrows to produce blood (refractory anemias) will be discussed elsewhere. Thus, the terms "aplastic" and "hypoplastic" will be used in the strict sense.

In many cases, the cause of the marrow aplasia can be determined, usually by exposure to certain physical or chemical agents, but in others no causative factor is evident. On this basis, these anemias are catalogued as secondary and primary respectively

PRIMARY (IDIOPATHIC)

Congenital Hypoplastic Anemia

Anemia of varying severity may develop from a few days to several months after birth without evidence of erythroblastosis or abnormal hemolysis. The red cells are of usual size, shape, and hemoglobin content, and the reticulocyte count is not increased over normal. The leukocyte and thrombocyte counts are not reduced in proportion to the erythrocytes, and may even be normal.

The hematopoietic cells in the bone marrow are decreased in number in proportion to the severity of the anemia, especially the red cell progenitors. They are loosely and haphazardly scattered at first (Fig 56), later being compactly arranged between the fat cells which come to occupy much of the marrow space. Most of the nucleated red cells are found in the late erythroblastic and normoblastic stages of development. Cells of the granulocytic series and megakaryocytes display no qualitative changes.

Transfusions of whole blood or red cell concentrates often maintain life for many months, and I have seen several babies require less and less blood until finally their marrows became adequate to support normal blood levels. Others fail to show any signs of regeneration and die despite repeated transfusions, usually from intercurrent infection.

Fanconi's syndrome is a form of congenital hypoplastic anemia which has a familial incidence. It is associated with pigmentation of the skin, hypogonadism, and developmental defects such as hypodactylia. The patients present a chronic aregenerative type of anemia with occasional showers of reticulocytes and nucleated red cells. There is usually an associated leukopenia and thrombocytopenia.

Acquired Hypoplastic Anemia

Certain persons, mostly women in the third to fifth decade of life, habitually show a moderate degree of anemia, with red cells ranging from 3,000,000 to 3,500,000 per cu mm. and hemoglobin in proportion. Neutrophils are also reduced in number. The thrombocyte count is occasionally decreased, but not markedly so.

These patients complain only of tiredness, and are frequently regarded as lazy or psychoneurotic. Study of the bone marrow, however, discloses a distinct decrease in hematopoietic tissue over normal for the age (Fig. 57) This can only be evaluated by section of aspirated

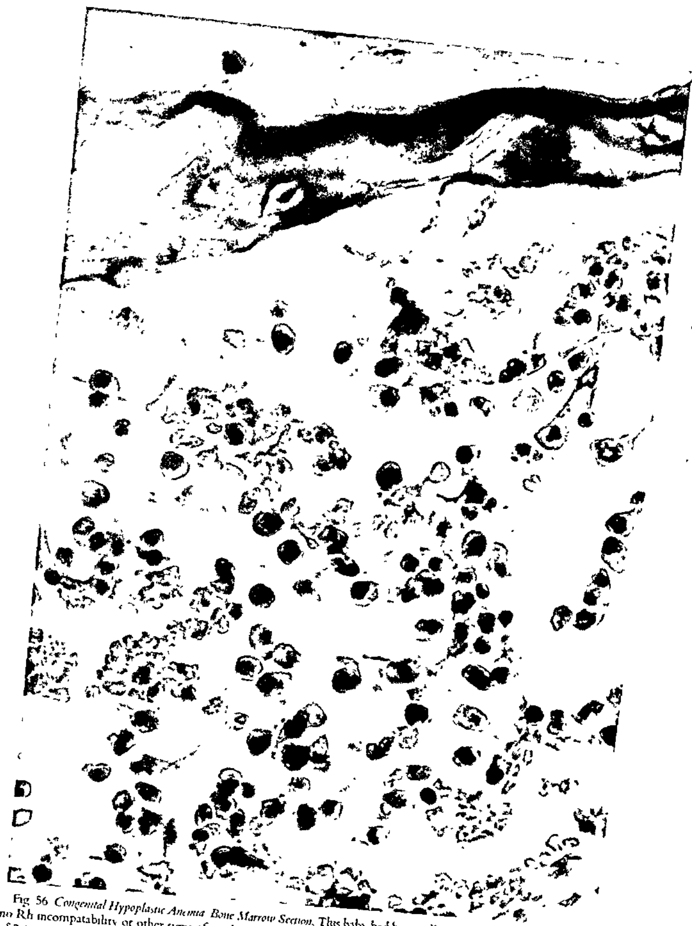


Fig 56 *Congenital Hypoplastic Anemia Bone Marrow Section.* This baby had been pallid since birth although ... no Rh incompatability or other signs of erythroblastosis fetalis. ... of 2,000,000 per cu mm ... months The marrow of a ... circumstances it would ha... marrow space would have ...

Fig 57 *Chronic Hypoplastic Anemia Bone Marrow Section* A sternal biopsy from a young woman with chronic aregenerative anemia and mild neutropenia discloses about half the normal complement of hematopoietic tissue to be expected at her age (× 1000)

Fig 58 *Idiopathic Aplastic Anemia Blood* The smear is thin, but the erythrocytes are normal in size, shape, and coloration The nucleated cell is a lymphocyte, very few neutrophils appearing in the peripheral blood Macrocytosis and poikilocytosis when present are favorable signs, as they indicate marrow regeneration (× 2100)

fragments of marrow or trephine biopsy; smears obtained by aspiration may be erroneously interpreted as poorly cellular from dilution with peripheral blood. No form of treatment is effectual, but spontaneous remissions sometimes occur. Some patients learn to enjoy their lassitude and to condition their families and associates to it, others are plied for years with injections of liver and iron, or are nauseated and constipated by the latest and most expensive "blood-building" capsule.

Idiopathic Aplastic Anemia

An unexplained disease observed most frequently in young adults, idiopathic aplastic anemia is characterized by an insidious onset, a relatively short course, and a fatal termination in the vast majority of cases. Progressive pallor and weakness are followed by hemorrhagic phenomena which may reach striking proportions and ulceration of mucous surfaces is prone to occur. There is usually high fever.

Significant Laboratory Data. Examination of the blood reveals a marked decrease in all of the formed elements The significant laboratory findings are shown in the following tabulation. The anemia is normocytic and normochromic in type (Fig. 58).

TABLE 13

LABORATORY FINDINGS IN IDIOPATHIC APLASTIC ANEMIA

Erythrocytes	Markedly decreased
Hemoglobin	Proportionately low
Erythrocyte diameter	Normal
Mean corpuscular volume	Normal
Volume index	Normal
Mean corpuscular hemoglobin concentration	Normal
Mean corpuscular hemoglobin	Normal
Color index	Normal
Saturation index	Normal
Erythrocyte resistance (hypotonic saline solution)	Normal
Reticulocytes	Decreased or absent
Leukocytes	Marked neutropenia, mild lymphopenia
Thrombocytes	Markedly decreased
van den Bergh reaction	Negative
Serum bilirubin	Normal
Bleeding time	Markedly increased
Coagulation time	Normal or slightly increased
Clot retraction	Little or none
Gastric analysis	Normal except for blood
Occult blood in stool	Positive

BONE MARROW. Hematopoietic tissue of the bone marrow is almost completely lost and the marrow spaces are filled with more or less bloody fat (Fig. 59). Smears of aspirated marrow show large fat droplets, many erythrocytes, and a paucity of nucleated elements A count of the nucleated cells reveals a large percentage of lymphocytes, plasmacytes, and macrophages (Fig. 60). The lymphocytic reaction may be so marked that the bone marrow examination is suggestive of lymphocytic leukemia (Fig. 61). Fibrosis of the marrow is occasionally observed in patients who have had an extended survival period; hemosiderosis resulting from many blood transfusions is usually prominent in these cases ("exogenous hemochromatosis") (Fig. 62). Cases of aregenerative anemia with normally cellular or hyperplastic marrow, frequently classified with the aplastic group, will be mentioned under the heading of refractory anemias.

Remission or recovery is preceded by regeneration of the marrow, apparently stemming from residual islands of erythroblasts and myelocytes, or from reticulum cells of the interstices (Fig. 63). In some instances, granulocytic proliferation appears first (Fig. 64), in others, erythropoiesis (Fig. 65). The peripheral blood shows slight macrocytosis during a regenerative phase, presumably due to an increase in reticulocytes, these cells being larger than mature erythrocytes.

Treatment. The rapid course of the disease can usually be checked by *transfusions of whole blood* or *red cell concentrates*, which should be continued as long as possible in the hope that regeneration of the bone marrow will come about. It is also necessary to administer *antibiotic agents* because of the low leukocyte levels, by this means otherwise rapidly fatal infections can be controlled, and mucosal ulceration minimized. A few patients recover completely, while others have survived for many months and occasionally several years, living largely on transfused blood.

SECONDARY

Causes of Secondary Aplastic Anemia

Exposure to Chemical Substances. The clinical and pathologic features of aplastic anemia

Fig 59 *Idiopathic Aplastic Anemia Bone Marrow Section* Marrow spaces normally occupied by hematopoietic tissue in the sternum of this twenty-year-old man are largely filled with fat Most of the other cells are lymphocytes and plasmacytes, with a scattering of residual nucleated red cells and myelocytes A few megakaryocytes remain (lower, right of center) There is a considerable extravasation of erythrocytes into the interstices (× 375)

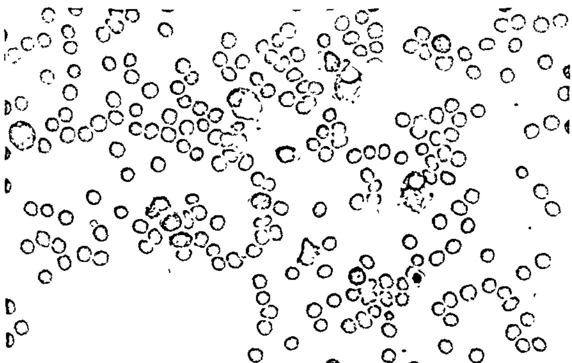


Fig 60 *Aplastic Anemia Due to Quinacrine Hydrochloride (Atabrine) Bone Marrow Aspiration* A large fat droplet occupies the lower left Most nucleated cells are lymphocytes and plasmacytes Several macrophages with engulfed red blood cells are shown (the patient had had blood transfusions) A solitary erythroblast is seen in the lower right (× 600) (Custer, R P : Am J M Sc, *212* 211 Courtesy of Lea & Febiger)

Fig 61. *Aplastic Anemia Due to Quinacrine Hydrochloride (Atabrine) Bone Marrow Section* The lymphocyte reaction may be so pronounced in some areas of the marrow as to simulate lymphocytic leukemia There is a rich intermingling of *plasmacytes and macrophages not usually seen in leukemia where the cell type is more likely to be purely lymphocytic* (× 120). (Custer, R. P.: Am J. M Sc. *212* 211. Courtesy of Lea & Febiger.)



Fig 62 *Aplastic Anemia Due to Quinacrine Hydrochloride (Atabrine) Bone Marrow Section.* A sternal biopsy performed *early in the course of the disease disclosed a fatty marrow. At autopsy ten months later, the marrow spaces of all bones* examined showed this extensive secondary myelofibrosis Hemosiderin-laden macrophages are conspicuous in the fibrillar mat. The patient had received more than 100 blood transfusions (× 550) (Custer, R. P · Am. J. M Sc., *212* 211 Courtesy of Lea & Febiger)

Fig 63 *Aplastic Anemia Due to Quinacrine Hydrochloride (Atabrine) Recovery Phase Blood* Erythrocytes show anisocytosis, and reticulocytes are beginning to appear Reticulocytes are distinguished in this bas relief print by their flatness and absence of central pallor. A metamyelocyte is seen in the upper left (× 2280)

Fig 64 *Aplastic Anemia Due to Quinacrine Hydrochloride (Atabrine) Recovery Phase. Bone Marrow Aspiration* The marrow had been virtually aplastic on the seventeenth day of the disease This biopsy on the seventy-second day shows marked regenerative activity, especially on the part of the granulocytic series, and the patient recovered (× 300). (Custer, R P Am J M Sc, *212* 211 Courtesy of Lea & Febiger)

which follows exposure to certain chemical agents do not differ essentially from those of the idiopathic type, except that the anemia is occasionally macrocytic. Chances of recovery are greater in the secondary group, especially if the cause is recognized promptly and removed. With the exception of lead, quinacrine hydrochloride (atabrine), and benzol, there seems to be little relationship between dose and duration of exposure, and the development and degree of anemia.

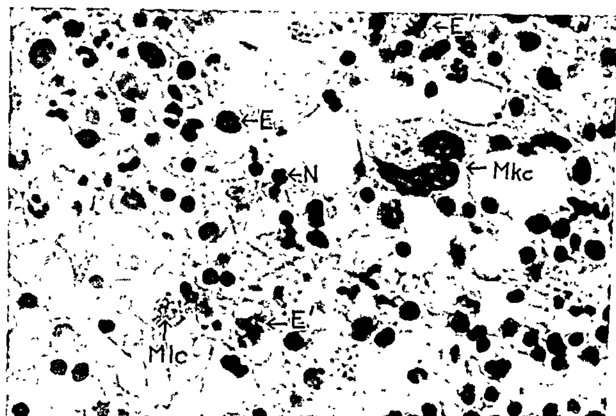


Fig 65. *Idiopathic Aplastic Anemia, Recovery Phase Bone Marrow Section* In contrast to a previously fatty state, this biopsy shows marked regeneration of hematopoietic elements, especially in the erythrocytic series, and resorption of fat (E, erythroblast, E', erythroblast in mitosis, N, normoblast, Mlc, myelocyte, Mkc, megakaryocyte) (× 1000).

Organic arsenical compounds, especially sulfarsphenamine, are perhaps most frequently responsible for the development of aplastic anemia. The number of reported cases is no measure of the actual incidence *Acute benzol poisoning* will produce aplastic anemia, whereas chronic exposure to small amounts produces anemia with a hyperplastic marrow and is thought to be followed in some cases by chronic granulocytic leukemia. *Quinacrine hydrochloride* (atabrine) employed in suppressive doses continuously for many months was responsible for at least fifty-seven deaths from aplastic anemia during the Second World War. The actual number of cases of aplastic anemia attributed to the use of quinacrine hydrochloride was infinitesimal, however, contrasted with anticipated morbidity and mortality from malaria had drug-suppressive therapy not been used. *Mustard gas* poisoning will produce aplastic anemia, but the nitrogen mustard compounds affect leukocytes for the most part, especially lymphocytes, erythrocyte and thrombocyte progenitors suffering to a lesser degree. *Trinitrotoluene*, *dinitrophenol*, and related compounds have been reported as causal agents, and more rarely *sulfonamides*, *colloidal silver*, *bismuth*, and *mercury*.

Exposure to Radiant Energy. The effects of radiant energy (roentgen rays, radium, radioactive isotopes) will be mentioned in more detail in another section (p. 174). Suffice to say here that, unlike most chemicals, their action on the blood-forming organs is directly related to dose and duration, and that heavy exposure

will cause depression of the bone marrow, with the development of anemia, leukopenia, and thrombocytopenia.

Marrow Exhaustion. Apart from starvation marrow (Figs. 27, 28, 37, and 259), exhaustion of the bone marrow is rarely encountered

Destruction of marrow with resulting aplastic anemia has been described as occurring in various infections, but I have seen this in only a few cases of septicemia caused by *Clostridium perfringens*, and even here the anemia was chiefly due to hemolysis (Fig. 191).

VII

DISPLACEMENT OF BONE MARROW
(Myelophthisis)

So-called "myelophthisic states" result from displacement of the bone marrow by a variety of tissues. The involvement may be focal or generalized Consideration will be given only those conditions in which serious dysfunction of the bone marrow results, with consequent crippling of blood formation. The process is generally slow, and the accessory blood-forming organs (notably the spleen and liver) gradually assume hematopoiesis. These organs become markedly enlarged, especially the spleen, where the histologic appearances are often similar to those of bone marrow, with megakaryocytes for some reason unusually abundant

The red cell level may be normal or increased for a long time, but anemia ultimately develops The anemia may be normocytic or macrocytic, and is sometimes marked by large numbers of nucleated red cells in the peripheral blood, associated with hyperleukocytosis and immaturity in cells of the granulocytic series (leuko-erythroblastosis). In other cases, the leukocyte count is low The thrombocyte count is variable, and giant thrombocytes and megakaryocytes may be found in the blood film

Diagnosis The diagnosis is usually difficult in this group of conditions, frequently impossible without the aid of bone marrow biopsy, although roentgenologic survey of the skeleton is often helpful. Attempted aspiration of bone marrow may result in a dry tap, and a button of bone should be removed with a trephine in such instances Patients presenting a mild anemia, leukopenia, and splenomegaly have had these symptoms loosely diagnosed as Banti's syndrome, and their spleens have been removed, only to find that a major blood-forming organ has been lost. The urge to remove a large spleen must be curbed until the cause of the splenomegaly has been determined.

PRIMARY (IDIOPATHIC)

Myelofibrosis

Diffuse fibrosis of the bone marrow without proliferation of bone (Fig. 66) is a rare condition which may develop without demonstrable cause. The blood may show a decrease in red cells, leukocytes, and thrombocytes, or there may be a leuko-erythroblastic blood picture. The spleen and liver are enlarged, owing to myeloid metaplasia. This is probably a variant of chronic granulocytic leukemia for reasons mentioned in the following section.

Myelofibrosis may also be a sequel of longstanding aplastic anemia where hemosiderosis of the marrow has followed many blood transfusions (exogenous hemochromatosis) (p 76, Fig. 62). The blood picture in these cases is aregenerative in type, and there is little or no myeloid metaplasia in the spleen and liver.

Osteosclerosis

Osteosclerosis is characterized by proliferation of cancellous bone in addition to fibrosis of the marrow spaces (Fig. 67) Any fundamental difference between this and myelofibrosis may be more apparent than real, especially when myeloid metaplasia is prominent. They have in common a relatively nonfunc-



Fig 66 *Primary (?) Myelofibrosis Bone Marrow Section* The patient was a white man of sixty-two, with a history of splenomegaly of at least ten years' duration Early blood studies showed a mild polycythemia, later a relatively normal blood count except for neutropenia, and during the last three years an anemia controlled by blood transfusions required at increasingly shorter intervals The anemic phase was marked by a leuko-erythroblastic blood picture Autopsy disclosed marrow fibrosis of all bones examined, and extensive myeloid metaplasia of the spleen and liver. The section shown is taken through a vertebral body, hematopoietic tissue is completely replaced by fibrous tissue containing hemosiderin-filled macrophages The iron pigment results from the breakdown of transfused blood (× 330)



Fig 67 *Primary (?) Osteosclerosis Bone Marrow Section* Five years prior to her admission to our hospital, this fifty-four-year-old white woman was subjected to splenectomy, the diagnosis of Banti's syndrome having been made on the basis of splenomegaly, mild anemia, leukopenia, and thrombocytopenia Our blood studies showed moderate anemia and marked leuko-erythroblastosis, with considerable immaturity of neutrophils Sternal aspiration was unsuccessful, and tissue from the trephine biopsy pictured here presented tremendous proliferation of cancellous bone with fibrosis of the marrow spaces Roentgenologic study of the entire skeleton disclosed marked radiopacity of all bones The patient lived three years longer, maintained on blood transfusions, and autopsy revealed advanced myeloid metaplasia in the enlarged liver and elsewhere Sections of the extirpated spleen showed the same changes (× 330)

tioning bone marrow, marked splenomegaly and moderate hepatomegaly, and occasionally a leuko-erythroblastic blood picture, although leukopenia is more characteristic.

This general category should probably include a group of closely related, if not identical, conditions which have been variously termed *sclerosing myelosis, osteosclerotic leukemia, leukanemia, aleukemic megakaryocytic myelosis, chronic nonleukemic myelosis, agnogenic myeloid mataplasia, myeloid megakaryocytic hepatosplenomegaly*, and so on I am reasonably sure that virtually all of the cases so designated are atypical forms of chronic granulocytic leukemia, judging from descriptions in the literature and periodic observation of a number of cases in my experience For example, Fig 68 illustrates early bone marrow hyperplasia coincident with a rising neutrophil count and progressive splenomegaly, all typical of chronic granulocytic leukemia. A sternal biopsy two years later disclosed bone proliferation and partial fibrosis of the marrow spaces, while at autopsy, seven years after the onset of the illness, all bones were densely sclerotic, resembling the marrow shown in Fig. 67. The spleen, liver, and lymph nodes were the seat of striking myeloid metaplasia, shared to a lesser degree by other tissues. Likewise, the sternal bone marrow pictured in Fig 69 is from a patient with classical clinical and hematologic features of chronic granulocytic leukemia, the history indicating that the disease had existed for seven or eight years Subsequent studies showed a progressive increase in number of nucleated red cells in the blood, as well as occasional megakaryocytes and giant thrombocytes, concomitant with advancing sclerosis of the bone marrow. We lost track of the patient, and no autopsy was made.

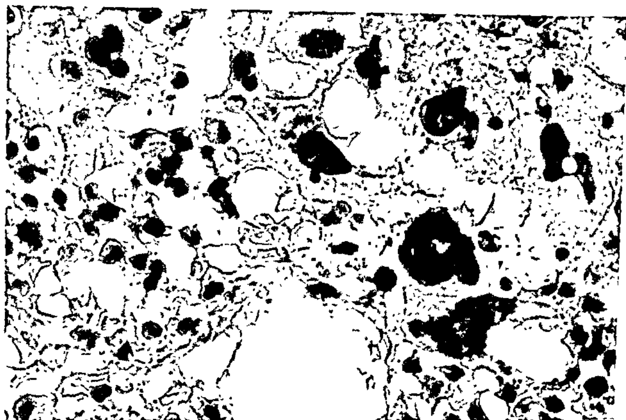
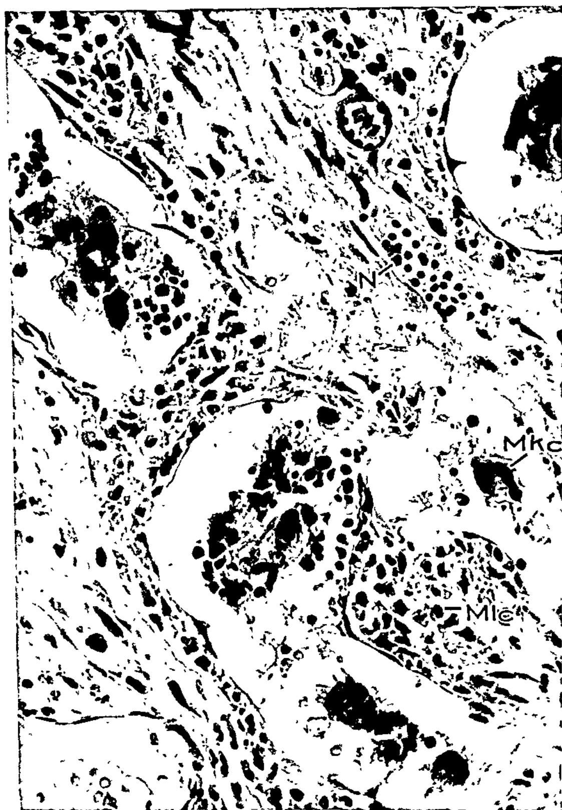


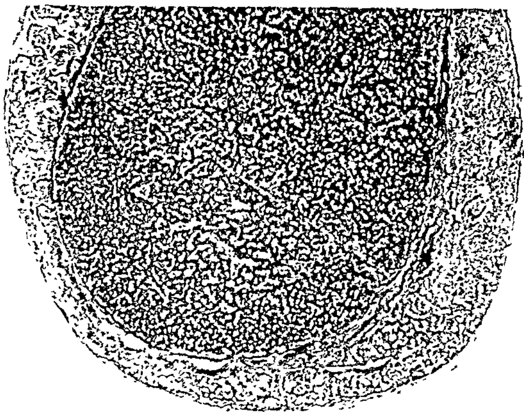
Fig 68 *Osteosclerosis, Early Sternal Biopsy Bone Marrow Section* This biopsy was performed on a patient showing an increasing neutrophil count and progressive splenomegaly The marrow shows active proliferation, especially of neutrophils and megakaryocytes, and pre-existing fat is being reduced to small droplets; no fibrosis is evident Over the next seven years, osteosclerosis developed, and the terminal picture of the bones was identical to that shown in Fig 67 (× 1000)



[illegible] red cells, and cells of the granulocytic series lie in the distended blood sinuses. Later there was a progressive increase in circulating erythroblasts and normoblasts, with occasional megakaryocytes and giant thrombocytes (× 650)

Other Bone Diseases of Unknown Cause

About one-fourth of the cases of *osteopetrosis* (marble bone disease, *Albers-Schönberg disease*) display evidence of myelophthisic anemia This is a congenital and probably familial disease in which the marrow is replaced by compact bone and is indistinguishable from the cortex (Figs. 70 and 71). A pericortical zone of normal bone often develops from the periosteum in which the marrow spaces are filled with active hematopoietic tissue (Fig. 70). The



fferentiation between cortex and medulla e. The peripheral zone is formed by rela- marrow spaces containing active hem- iois, Chicago) (× 10)

process is limited to long bones (Fig. 72). Splenomegaly is frequently found.

Anemia may occur in the later stages of *osteitis deformans* (Paget's disease) when a number of bones are involved, especially vertebrae, and the marrow spaces extensively fibrosed (Fig. 73). The myeloid cavity of long bones is usually better preserved and the marrow shows reactive hyperplasia when the flat bones are generally affected by the disease. The large soft bones present a diagnostic roentgenologic appearance.

SECONDARY DISPLACEMENT

Osteitis Fibrosa Cystica

Hyperparathyroidism effects a profound alteration in the skeleton, primarily through demineralization of bone. Subsequently there is partial loss of the decalcified bone matrix followed by replacement fibrosis. The fibrous reaction may be so widespread in advanced cases that myelophthisic anemia develops. As the anemia is a late feature of the disease, it presents no diagnostic difficulties. Anemia in hyperparathyroidism may also result from chronic renal failure, because a significant number of these patients have renal calculi and pyelonephritis

Leukemia and Erythremia

Displacement of the bone marrow by the neoplastic cells of leukemias will be considered in Chapter XIII Erythrocytosis may be a feature of osteosclerosis and myelofibrosis for varying periods of time, and may be confused with true erythremia (polycythemia vera).

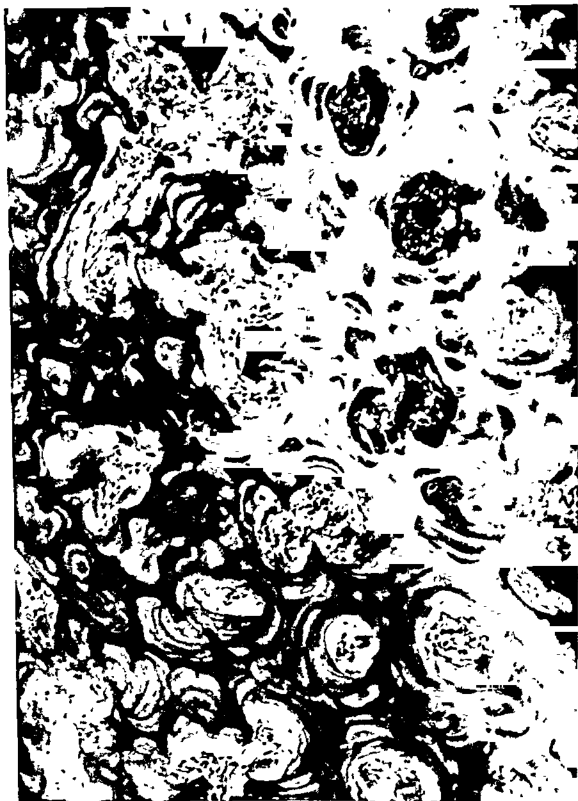


Fig 71 *Osteopetrosis (Marble Bone Disease) Section of Tibia* Higher magnification of the bone shown in Fig 70 shows the peculiar whorling and lamination in closely packed trabeculae The tiny marrow spaces contain loose fibrous tissue, blood vessels, and a sparse scattering of blood cell progenitors (Slide, courtesy of Dr. Granville Bennett, University of Illinois, Chicago) (× 250)

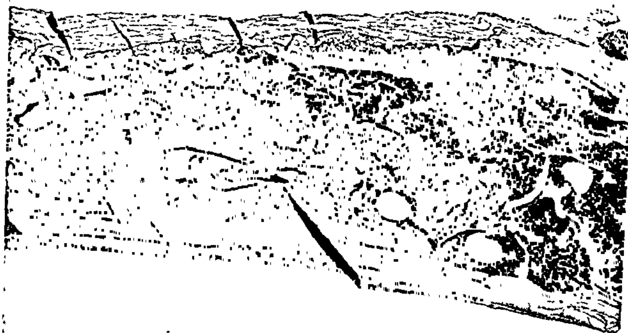


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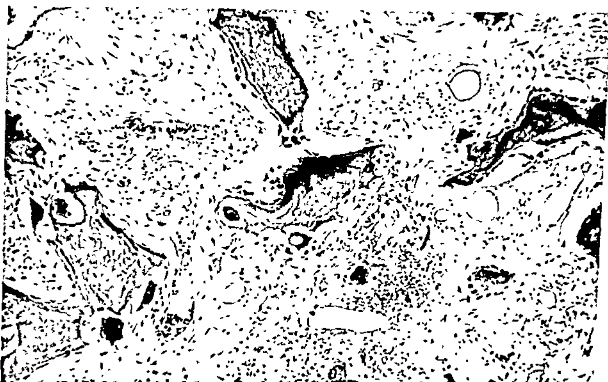


Fig. 73. *Osteitis Deformans (Paget's Disease) Section of Vertebra* The typical bulky bone trabeculae with mosaic pattern are not shown in this field, which was selected to emphasize the advanced degree of myelofibrosis present in this case, resulting in marked myelophthisic anemia (× 150)

Tumors

If one includes the leukemias and erythremia, it is possible to generalize that tumors may arise from any one of the cellular components of the bone marrow In addition, the histogenesis of Ewing's tumor is not clear. Tumors of fat, nerves, blood vessels, and fibrous tissue are of little importance in the field of hematology. Multiple myeloma and the lymphomas, along with Ewing's tumor, comprise the marrow neoplasms of greater significance.

The precise cause of anemia in malignant disease is not clear (see p. 151). Certainly displacement of bone marrow accounts for it in a relatively small proportion of cases.

Multiple myeloma is a tumor arising in the marrow of several or many bones, the growth foci apparently being independent of one another rather than representing multiple metastases. Solitary myelomas have been mentioned in the literature, but in the several cases that I have seen, myeloma cells could be demonstrated in the marrow of seemingly uninvolved bones. The tumors are usually found in bones which normally contain cellular marrow in the adult, i e. those of the trunk and skull. Soft tissues may be involved as a result of direct extension of the tumor, by metastasis, and possibly through autochthonous growth from reticulum cells.

Multiple myeloma is encountered most frequently beyond the age of forty, although a few cases have been described in children. Clinically, pain referable to bone is the most constant complaint. Occasionally anemia, hemorrhage, or symptoms of renal disease may be the presenting features. Pathologic fractures are apt to occur, with consequent deformities; in the event of vertebral collapse, neurologic manifestations may be prominent. The classical roentgenologic finding of "punched-out" lesions of the bones is frequently absent. Sometimes it is possible to demonstrate only a few vaguely defined areas of radiolucency, or the skeleton may show merely a diffuse osteoporosis of varying degree. I have seen one case in which all bones appeared quite normal in the films for some months after the diagnosis had been clearly established (Figs. 74, 75, 76, 77).

Anemia is not necessarily present in proportion to the extent of skeletal involvement, unless marrow replacement is extremely widespread. It is usually normocytic in type, occasionally macrocytic. Rarely there is no anemia. The leukocyte count is inconstant, seldom reaching very low or very high levels. The differential count is not significant except when there is spillage of tumor cells into the peripheral blood (Fig. 78). Thrombocyte counts are usually within normal limits and are apparently unrelated to the bleeding tendency in some patients Hyperproteinemia has been observed in a considerable proportion of patients, owing solely to an increase in globulins; in fact, the albumin level of the plasma is sometimes below normal. This increase in globulins is apparently responsible for the marked clumping and rouleaux frequently noted in the red blood cells, often to the extent that it is impossible to perform an erythrocyte count or prepare a smooth blood film (Fig. 74). Warming the diluting fluid sometimes loosens the clumps sufficiently to permit counting, and for this reason some observers believe that the phenomenon is due to the presence of cold agglutinins. Whatever the cause may be, observation of such striking rouleau formation should stimulate further investigation In three patients in my series, this observation during routine blood counts led to the correct diagnosis of myeloma, the tumors having been quite unsuspected. Other findings attributed to the hyperproteinemia are the extremely rapid sedimentation rate, damage to the kidneys, and thrombosis of small blood vessels. High serum calcium levels have been noted in about 50 per cent of cases, sometimes associated with metastatic calcification of the soft tissues Serum phosphorus is normal or increased, a point of distinction from the hypercalcemia of osteitis fibrosa cystica. Over half of the patients excrete Bence Jones protein in their urine, some intermittently, others constantly. Bence Jones proteinuria occurs most frequently in cases where the tumor cells are of the large, poorly differentiated type.

Fig 74 *Multiple Myeloma Blood.* Clumping of red cells is sometimes an early clue to the diagnosis of multiple myeloma. In this case, the only complaint was low back pain, and roentgenologic study did not disclose any bone changes Observation of the pseudorouleau formation in the blood smear led to determination of plasma proteins (albumin, 2 gm , globulin, 9 gm per 100 ml) and the finding of Bence Jones protein in the urine Result of the sternal puncture is shown in the two following pictures (× 2100)

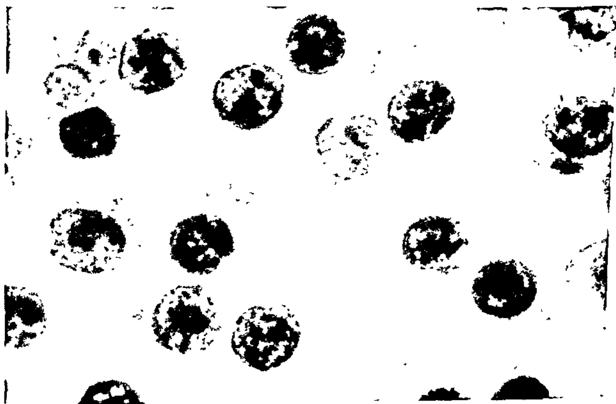


Fig. 75 *Multiple Myeloma Bone Marrow Smear* A fairly high percentage of the aspirated cells in this case are neoplastic. They are relatively small and resemble mature plasmacytes (× 2100)

[illegible]
as [illegible]
are [illegible]
and [illegible] at the time of biopsy (× 1000)

Fig 77 *Multiple Myeloma Bone Marrow Section, Late* At autopsy, extensive areas of rarefaction were found throughout the skeleton (same case as Figs 74, 75, and 76) Hematopoietic tissue and fat have been completely displaced by tumor (× 1000).

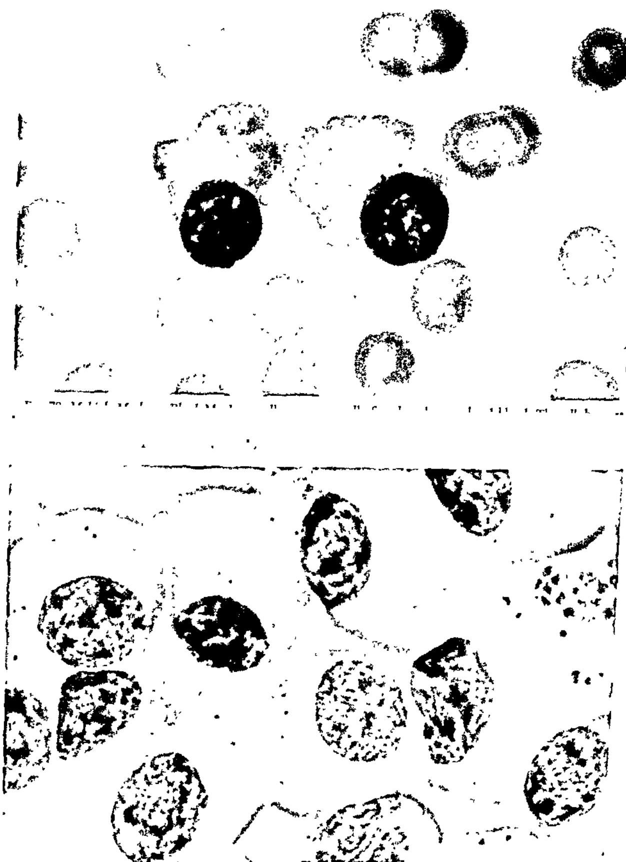


Fig 79 Multiple Myeloma Bone Marrow Smear (same case as *Fig 78*) Tumor cells in the marrow of this patient exhibit a still greater degree of anaplasia than those found in the peripheral blood, being larger and having a more vesicular nucleus, which is sometimes centrally placed (× 2100)

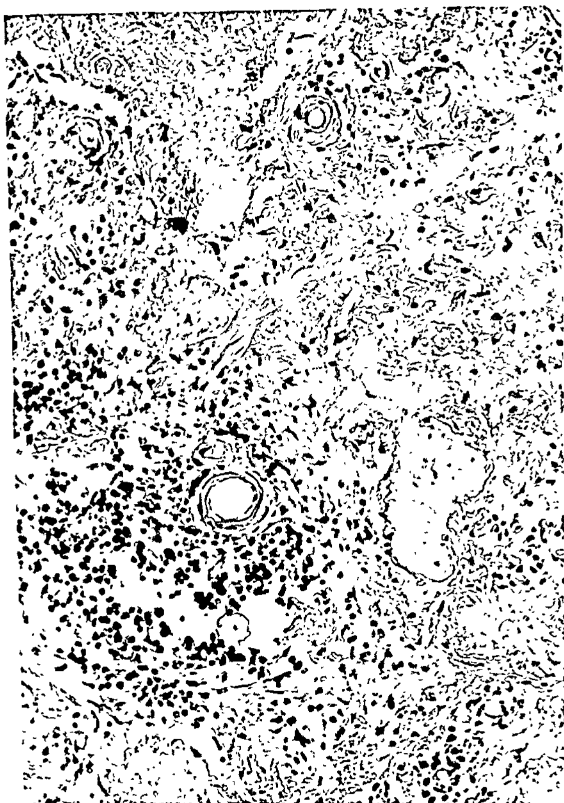


Fig. 80 *Multiple Myeloma, Effect of Radiant Energy. Bone Marrow Section.* This patient had been subjected to intensive treatment with high-voltage roentgen rays. Much of the tumor has been destroyed, and the marrow spaces are partly replaced by fibrous and osteoid tissue. An area of apparently viable tumor is seen in the left lower quadrant of the picture. Note the thickening of blood vessel walls (× 350).

The myeloma cells, in common with the cells of virtually all malignant tumors, show variable degrees of differentiation from patient to patient. In the more anaplastic forms, they are large (30 to 35 μ) with abundant bright blue cytoplasm and no perinuclear pallor, the eccentric or centrally placed nucleus being moderately large and vesicular, some containing nucleoli (Fig. 79). Occasionally there is a striking resemblance to reticulum cells. Cells of the better differentiated tumors are smaller (10 to 15 μ), and their likeness to plasmacytes *is pronounced, save for a finer* chromatin structure of the nucleus (Figs. 75, 76, 77). *All gradations between the two extremes* may be observed, although in individual *patients, the cells maintain a fairly uniform* quality. In common with plasmacytes, the *myeloma cells* are frequently *binuclear*. There is little or no ground for recognizing myeloblastic, lymphoblastic, erythroblastic, or megakaryoblastic forms as described in the literature. Such designations have apparently resulted from errors in identification of partially differentiated plasmacytic myeloma cells.

Final diagnosis rests with the finding of tumor cells in the biopsy. Sometimes several marrow aspirations from different sites (sternum, iliac crest, vertebral spinous process) are required in early cases where tumor foci are not evident on roentgenograms. *The presence of typical plasmacytes in considerable numbers in a marrow aspirate must not be regarded as conclusive evidence of myeloma,* as this may be associated with chronic granulomatous inflammation. The cells must display qualitative deviation from the normal, especially in the nucleus, before they can be clearly identified as neoplastic.

Considerable destruction of tumor can be effected by high-voltage *roentgen ray therapy* in some cases (Fig. 80), but the ultimate prognosis is not significantly altered. *Symptomatic* relief is sometimes obtained by the use of stilbamidine, pentamidine, or antimony; myeloma cells persist in the marrow, however, and *frequently develop metachromatic inclusion* bodies containing ribose nucleic acid. It has *been reported that* ethyl carbamate (urethane) is a still more effective therapeutic agent. The average length of life after recognition of the tumor is about two years, although certain patients may go through a *series of remissions* and relapses to live very much longer.

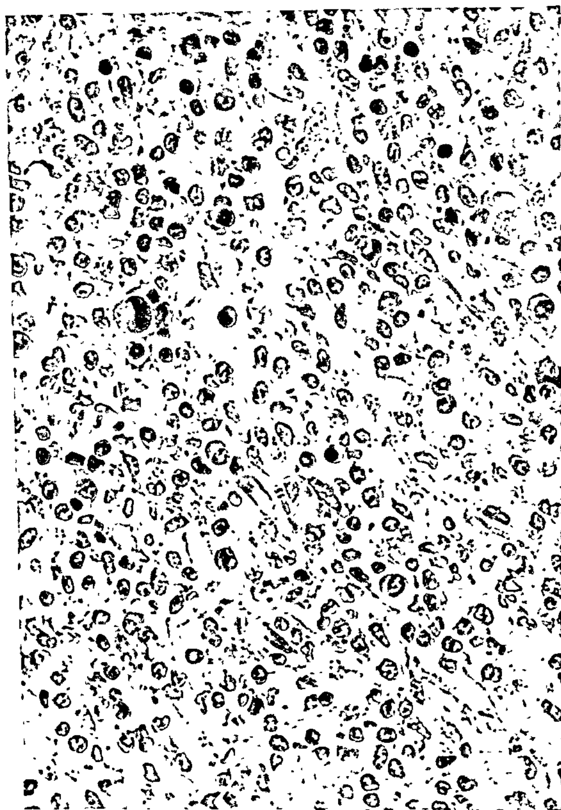
The *lymphomas may arise primarily in bone* marrow and be confined there for a time, but *seldom do. Marrow involvement is more frequently* part of a more or less generalized *lymphomatosis. The affected bones show* a marrow filled with gray-white fleshy tumor which *usually destroys the trabeculae and erodes the* cortex, sometimes resulting in a pathological *fracture. Pain may call attention to the bone* lesions, which are readily demonstrable in roentgenograms; in other cases, unsuspected spread of tumor through the marrow may be disclosed at autopsy.

Anemia is sometimes a conspicuous feature of the lymphomas. When coupled with leukopenia and thrombocytopenia, one may suspect rather extensive marrow displacement (Figs. 81, 82, 83). Acute hemolytic anemia is occasionally encountered. The leukocyte picture is exceedingly variable. Patients with Hodgkin's granuloma frequently show a neutrophilic or eosinophilic leukocytosis (Figs. 170, 171), monocytosis, and absolute lymphopenia. Monocytosis and the appearance of monocytoid tumor cells have been observed in the peripheral blood in some cases of follicular lymphoblastoma (Fig. 84).

The interrelationship of the lymphomas was established by our study of 1300 patients, many by serial biopsies and autopsy.* Transitions from one histologic type of tumor to another occurred in nearly 40 per cent of patients, and *several different microscopic patterns often* were present in the same person, even in the *same lymph node. The transitions actually* observed are shown in Fig. 86. It was concluded *that there is only one malignant tumor* of lymphatic tissue, a malignant lymphoma, which *can adopt a number of* histologic appearances, pure or mixed, and that considerable fluidity exists between these different forms.

The action of radiant energy, nitrogen mustards, and bacterial polysaccharides on the lymphomas is much the same in causing temporary regression of the tumors, varying with each

* Am. J. Med. Sc., 216:625, 1948



cytes, reticulum cells, fibroblasts, some neutrophils and eosinophils A Reed-Sternberg giant cell lies to the left (× 1000).

Fig. 82 *Malignant Lymphoma, Reticulum Cell (Hodgkin's) Sarcoma Type. Bone Marrow Section.* This tumor was apparently primary in the proximal end of the tibia of a forty-four-year-old man Most of the cells are large, variable in shape, and have vesicular nuclei, they lie in intimate relationship to a delicate reticulin network which was demonstrated by silver impregnation methods (not visible in this hematoxylin-eosin preparation). A partially destroyed bone trabecula is seen in the center (× 1000).

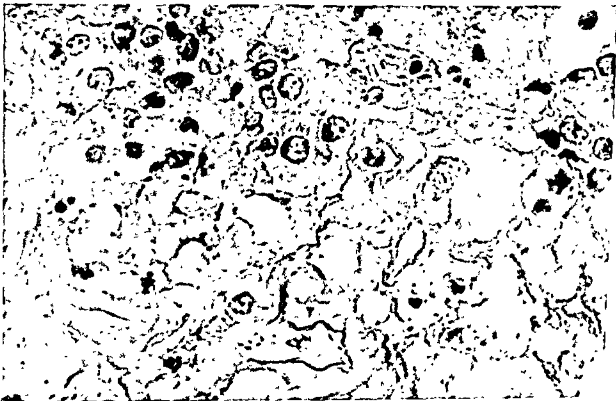


Fig 83 *Malignant Lymphoma, Reticulum Cell (Hodgkin's) Sarcoma Type Effect of Radiant Energy. Bone Marrow Section* The same tumor shown in Fig 82 following high-voltage roentgen-ray therapy Most of the cells have completely disintegrated, leaving a fibrillar framework. A group of possibly viable cells is shown in the upper left (× 1000)

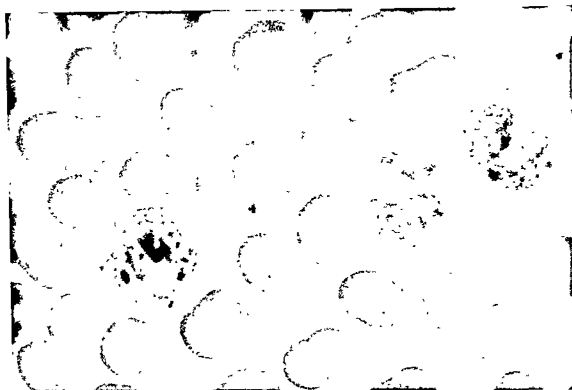


Fig 84. *Malignant Lymphoma, Follicular Lymphoblastoma Type. Blood* Monocytosis and monocytoid tumor cells were occasionally noted in the blood. A well-differentiated monocyte is seen to the right, while the other two cells are regarded as circulating tumor cells (× 2100)

patient in effectiveness rather than with the particular type of lymphoma. An exception is the follicular lymphoblastoma which is particularly radiosensitive for an unpredictable period of time Acquired radioresistance of a tumor may sometimes be overcome by treatment with either nitrogen mustards or bacterial polysaccharides

Diagnosis of the lymphomas is necessarily dependent on the histologic examination of affected tissue, the site of biopsy varying with the patient.

Ewing's tumor (endothelial myeloma) as a pathologic entity remains in doubt This much can be said, that it is a nonosteogenic, radiosensitive tumor (Fig. 91) occurring usually in long bones of young persons, that it tends to elevate the periosteum and invade surrounding soft tissues, and that it is composed of relatively small, round cells growing in diffuse sheets, gard Ewing's tumor as a metastatic neuroblastoma in bone. It is not within the scope of this book to discuss the merits of this view. The blood of these patients shows pancytopenia when the tumor is widespread and bone marrow displacement is extensive. Anemia is frequently observed also when the tumor is relatively localized.

Metastatic, or invading, tumors in bone marrow may provoke a variety of reactions, depending largely on the desmoplastic or chemical nature of the primary tumor For example, the tumor shown in Fig. 92 was primary in the stomach where it formed a highly cellular growth with a minimum of fibrous stroma, at the sites of bone metastases, the bone underwent rarefaction and resorption, and the adjacent hematopoietic tissue showed erythroblastic hyperplasia and eosinophilia, but no fibrosis. In Fig. 93, the marrow is extensively fibrotic, the primary tumor having been a small scirrhous carcinoma of the breast. Other tumors, notably carcinoma of the prostate, evoke bulky proliferation of the affected bone

Fig 85 *Follicular Lymphoblastoma Bone Marrow Section* A white woman of sixty-three years had enlargement of superi [illegible] There [illegible] later Autopsy disclosed disseminated follicular lymphoblastoma involving lymph nodes, spleen, bone marrow, liver, and lungs, with a more diffuse infiltration of heart, adrenals, kidneys, ovaries, and uterus Viewed alone, the marrow picture would also be suggestive of nodular involvement in lymphocytic leukemia, although the foci are much more sharply defined here. (Cohen, S E, and Bergstrom, V. W.: Am J Clin Path : *16* 22. Photomicrographs by courtesy of Dr Victor W Bergstrom, Kilmer Memorial Laboratory, Binghamton City Hospital, Binghamton, N. Y)

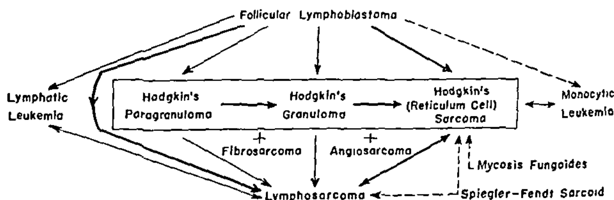


Fig 86 *Interrelationship of Lymphomas* The solid lines indicate transitions actually observed in our series in sequential biopsies or between biopsy and autopsy The heavy lines show the changes most frequently seen, the lighter the more unusual ones Dotted lines indicate transitions not seen in this particular group of patients, but observed or recorded elsewhere

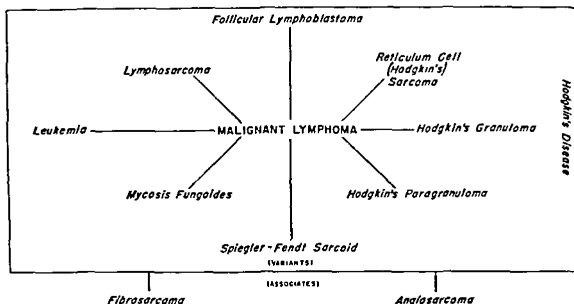


Fig 87. Variants and associates of malignant lymphoma.

Fig 88 *Malignant Lymphoma, Lymphosarcoma Type. Bone Marrow Section* There is virtually complete replacement of hematopoietic tissue by tumor composed of round cells of uniform size A degenerated megakaryocyte in the upper center is the only identifiable remnant of marrow tissue (× 500)

Fig 89. *Malignant Lymphoma, Lymphosarcoma Type. Effect of Radiant Energy. Bone Marrow Section* The same tumor pictured in Fig 88 The patient died one week after roentgen ray therapy was begun Large areas of the tumor are already necrotic (× 500)

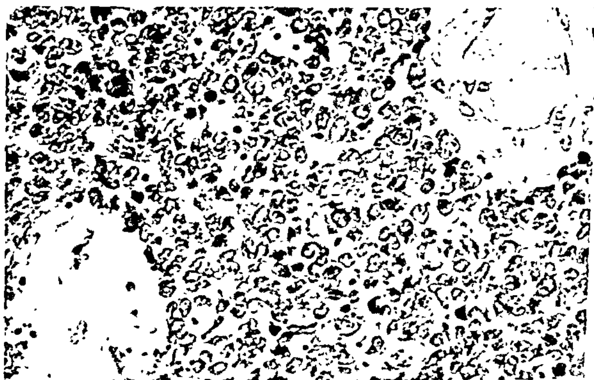


Fig 90 *Ewing's Tumor Bone Marrow Section* The tumor forms homogeneous sheets of cells traversed by coarse fibrous trabeculae which carry the larger blood vessels The cells are relatively uniform in size and shape, their cytoplasm being scanty and their nucleus moderately chromatic (× 800)

(Fig. 94); this probably results from the action of chemical products of the tumor, for example, acid phosphatase in prostatic carcinoma.

The bones generally involved by tumor metastases are those in which hematopoiesis is active throughout life, and this is usually reflected in the blood picture. Anemia may be of the myelophthisic type, associated with a decrease in neutrophils and thrombocytes, or far less frequently leuko-erythroblastosis may develop In the latter case, the peripheral blood is flooded with nucleated red cells and cells of the granulocytic series, of which many are myelocytes or still younger forms. Patients with leuko-erythroblastosis due to space-taking lesions of the marrow are not necessarily anemic and may even be polycythemic; this situation does not seem to be dependent on the extent of marrow involvement as does myelophthisic anemia.

In some cases, tumor may be quite unsuspected and disclosed only by biopsy of the bone marrow (Figs 92, 93, 94) Roentgenologic survey of the skeleton, especially planagrams of the sternum, are often helpful in selecting the site of biopsy.

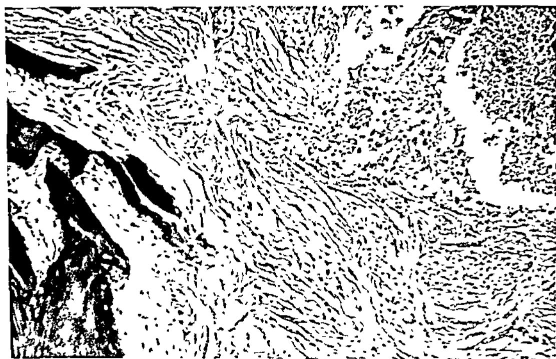


Fig 91 *Ewing's Tumor. Effect of Radiant Energy. Bone Marrow Section* The specimen was a leg which was amputated soon after a course of high-voltage roentgen-ray therapy had been completed The tumor seems to have been destroyed, its site marked by fibrous tissue deposits and areas of necrosis as seen in the upper right of the picture. Despite this, tumor reappeared in other sites (× 150).

Storage Diseases

These poorly understood conditions are characterized by the accumulation of lipids in cells of the reticulo-endothelial system. The cells multiply, with the result that the affected organs are enlarged, sometimes enormously, bones are eroded, and the bone marrow partially displaced. It is not yet clear whether the disturbances are primarily in the metabolism of lipids, with the cellular proliferation being merely a reactive one, or whether the defect is in the metabolic activity of the reticulo-endothelial cells proper.

Gaucher's Disease. This disease usually becomes manifest in early life, occasionally later, with an average duration of about twenty years. It is transmitted as a mendelian dominant character, with carriers frequently unrecognized except by biopsy.* The infantile and neurologic forms offer a less favorable

* Blood

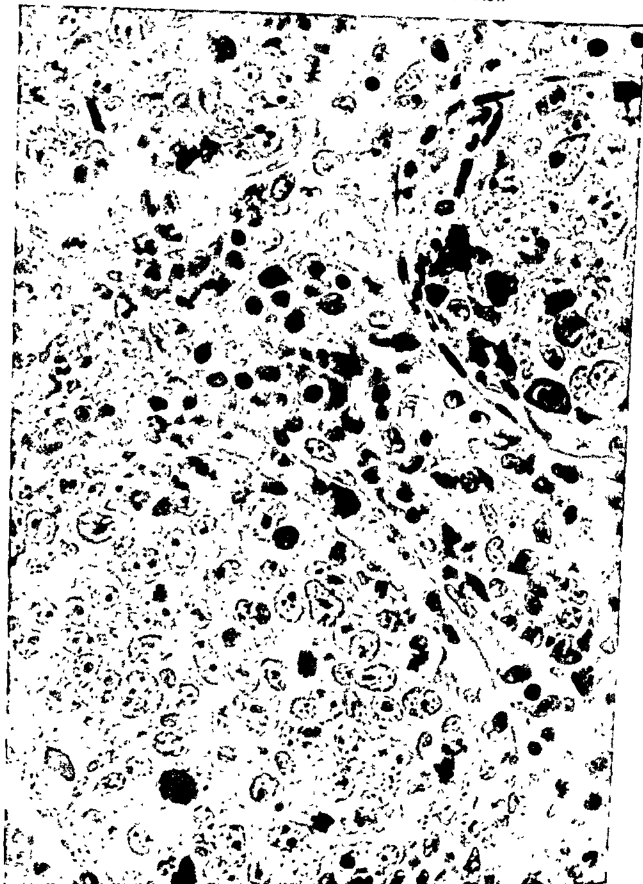


Fig. 92. *Secondary Carcinoma. Bone Marrow Section.* This sternal biopsy shows blood sinuses of the marrow greatly distended by masses of anaplastic epithelial tumor. The patient had a severe macrocytic anemia and gastric anacidity, and was thought to have pernicious anemia. Subsequent study disclosed a primary carcinoma of the stomach, located high in the fundus, which had not been seen in an earlier roentgenologic survey. (× 1000)



[illegible]

not palpable in the center of a large breast, and was discovered at autopsy It had metastasized only to bone (× 175)



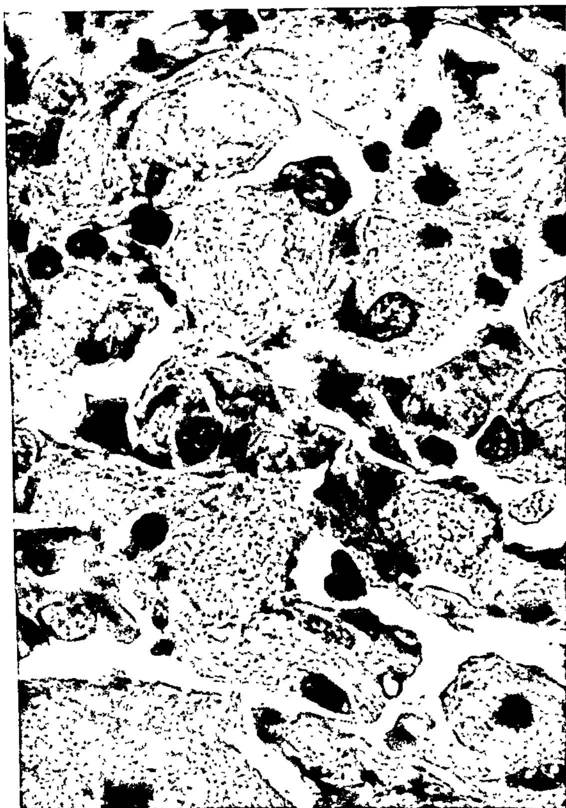
Fig 94 *Secondary Carcinoma Bone Marrow Section* Biopsy of a rib from a patient with advanced osteosclerosis involving the entire skeleton, he had a severe leuko-erythroblastic anemia Thickening of bone cortex and trabeculae, and fibrosis of the marrow spaces serve to displace all hematopoietic tissue A few tiny clusters of neoplastic epithelium constituted the only evidence of tumor, which was later found to be primary in the prostate gland (× 10) (Slide by courtesy of the Mt. Alto Veterans Administration Hospital, Washington, D. C)

Fig 95. *Gaucher's Disease Bone Marrow Section* Sternal biopsy performed on a nine-year-old girl shows large Gaucher cells loosely arranged among normal marrow elements, mostly red cell progenitors The Gaucher cells were faintly stained with Sudan III (× 1000).

Fig 96 *Gaucher's Disease Bone Marrow Section* The patient was a man aged about twenty-five years whose spleen was enormous The sternal biopsy shows extensive replacement of marrow by sheets of Gaucher cells, associated with considerable sclerosis which does not approach the degree often found in Hand-Schuller-Christian disease, however (Compare with Fig 104) (× 650)



Fig 97 *Gaucher's Disease Bone Marrow Smear* Aspirated marrow from the sternum contains many large cells with bulky, palely staining, faintly reticulated cytoplasm, and small generally eccentric nuclei The patient, a sixty-eight-year-old white woman, had an enormous spleen removed in 1919, and in 1933 suffered a pathological fracture of the neck of the left f [illegible]
and evidence of [illegible]
(May-Grunwald [illegible]



thrombocytopenia who was admitted to the hospital with the tentative diagnosis of Banti's syndrome (× 2280)

prognosis. Symptoms are generally related to the enlarged spleen which may attain great size. There may also be moderate enlargement of the liver and lymph nodes. The skin is frequently discolored from a yellow to gray-brown hue, and wedge-shaped pingueculae of the conjunctiva are often found in adults. Roentgenologic study discloses rarefying bone lesions in most cases, and pathological fractures have occurred. The tissues in general, but notably the spleen, liver, lymph nodes, and bone marrow, contain nests of so-called "Gaucher's cells" (Fig. 95), usually in alveolar arrangement, sometimes in diffuse sheets (Fig. 96). The Gaucher cells vary from 20 to 80 μ in diameter, and have one or several small, eccentrically placed nuclei. The bulky cytoplasm is faintly acidophilic, and is only lightly stained by Romanowsky technics (Fig. 97). A fibrillar cytoplasmic network (Fig. 98) can be demonstrated by Mallory's aniline blue-orange G method, the characteristic lipid (kerasin, a cerebroside or cerebroglycoside) being deposited between the meshes These fibrillae are often visible in smears or imprints with Romanowsky stains. The usual fat solvents will not dissolve kerasin, and it is at most only faintly stained with Sudan III. The cells also contain a few granules of hemosiderin.

Blood studies in some cases reveal a moderate degree of anemia, normocytic in type, without much evidence of regenerative activity of the marrow. There is generally an associated neutropenia and slight decrease in thrombocytes Gaucher cells are rarely encountered in blood films. In the osseous form of the disease, marrow displacement may be so extensive that the anemia, leukopenia, and thrombocytopenia are very marked. In other patients the anemia or panhematocytopenia is apparently due to "hypersplenism" (p 114), as evidenced by striking remission after splenectomy The diagnosis is readily made by aspiration of the bone marrow and recognition of the typical cells.

Niemann-Pick Disease This disorder occurs in infancy and has a predilection for the Jewish race It is far more rapid in its progress than Gaucher's disease, and the involvement of tissues is more widespread The patients rarely if ever survive the second year of life. They fail to gain weight normally, have gastro-intestinal disturbances, and develop striking enlargement of the spleen, liver, and usually lymph nodes. A cherry red spot in the retina near the macula, typical of amaurotic family idiocy, is sometimes observed. Osteoporosis may be demonstrated on roentgenograms. Tissue changes resemble those of Gaucher's disease except that the lipoid histiocytosis is much more extensive. The characteristic cell is from 20 to 80 μ in diameter and the cytoplasmic vacuoles are spherical (Figs. 99, 100). The lipid filling the vacuoles is a phosphatide (sphingomyelin) which accepts sudan dyes and Nile-blue sulfate and is soluble in the usual fat solvents.

Anemia is not usually severe, although hypochromia is sometimes marked, owing to general malnutrition and iron deficiency. Neutropenia, and relative or absolute lymphocytosis and monocytosis are frequently noted, as well as thrombocytopenia. The large lipid-bearing histiocytes occasionally enter the circulating blood. Splenectomy has effected temporary improvement for a short time in some cases, no change in others.

Eosinophilic Granuloma. These destructive granulomas of bone may be solitary or multiple and may affect any bone except those of the hands and feet, with a predilection for the skull, vertebra, ribs, pelvis, humerus, and femur. The lesions are noted mainly in children and young adults, especially males. Tenderness and swelling usually call attention to them, and roentgenograms disclose irregular areas of radiolucency which must be differentiated from primary and secondary tumors Tissue curetted from the bone defects is yellowish-brown and friable, often with areas of frank hemorrhage. The histologic appearances vary from broad sheets of macrophages suggesting tumor to areas in which the multiplicity of cell types (macrophages, lymphocytes, plasmacytes, fibroblasts, neutrophils, and especially eosinophils, Fig. 101) denote an inflammatory process. Multinucleated giant cells (Fig. 102) are characteristically found in either situation. Necrosis and hemorrhage may be prominent. As the lesions progress, the macrophages engulf fatty substances from the displaced mar-

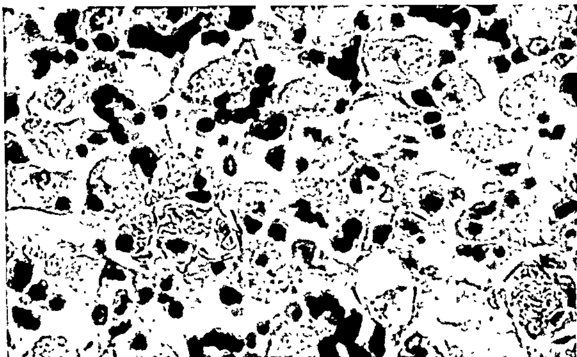


Fig. 99. *Niemann-Pick Disease Bone Marrow Section* This eighteen-month-old Syrian male infant had gained only 5 lb in weight since birth, and had exhibited weakness and a large abdomen since the age of three months The liver and spleen extended below the level of the anterosuperior iliac spines The blood showed a hypochromic anemia, increased resistance of red cells, lymphocytic leukocytosis, and reduction in thrombocytes Stools were large, foamy, [illegible]

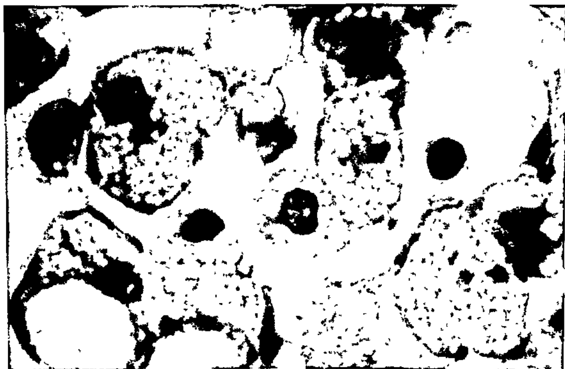


Fig 100 *Niemann-Pick Disease Bone Marrow Section* Section from the case illustrated in Fig 99 stained by Mallory's aniline blue-orange G method to accentuate the cytoplasmic vacuolization Compare these with the Gaucher cells shown in Fig 98 (× 2260) (Slide by courtesy of Dr Sidney Farber, Children's Hospital, Boston)

Fig. 101. *Eosinophilic Granuloma of Bone Bone Marrow Section.* Swelling and tenderness called attention to an osteolytic lesion in the tibia of a young man. The tissue shows dense aggregates of eosinophils distributed irregularly. The background is formed by sheets of histiocytes. Interspersed giant cells are not included in this field (× 1000).

Fig 102 *Eosinophilic Granuloma of Bone Bone Marrow Smear* Same case as pictured in Fig. 101 Smears of the biopsied material disclosed large numbers of histiocytes and a scattering of multinucleated giant cells, with relatively few eosinophils in the particular fragment used in preparing the smears Contrast this with sectioned material shown in Fig. 101 (× 1500)

Fig 103. *Hand-Schüller-Christian Disease Bone Marrow Section.* Tissue removed from an area showing rarefaction contains many lipoid-laden histiocytes Lymphocytes and eosinophils were also present, but are not evident in this field (× 1000)

Fig 104 *Hand-Schüller-Christian Disease Bone Marrow Section* This case was of longer duration than the one pictured in Fig 103 Here the tissue has more of a granulomatous character, with fibroblasts more conspicuous and the foam cells less prominent (× 1000)

row, and large foam cells may predominate, to resemble the lesions of Hand-Schuller-Christian disease very closely. Finally, fibroblastic proliferation may result in spontaneous regression of the lesions, and the residual scars are sometimes indistinguishable from fibrous dysplasia of bone. Laboratory studies, apart from showing an occasional mild eosinophilia, are not helpful in establishing the diagnosis, and microscopic examination of curettings from an affected bone are essential.

Hand-Schüller-Christian Disease. This disorder is classically pictured as showing "defects in membranous bones, exophthalmos, and diabetes insipidus." Actually, this syndrome is based only upon the fact that in about 85 per cent of cases the bones around the sella turcica and orbit are involved to a major degree. Deafness and loose teeth result from deposits of granulomatous tissue in the temporal bone and alveolar processes respectively. Any tissue may be affected, and biopsy of the bone marrow may show the lipophagic granulomatosis in areas not associated with bone rarefaction (Fig. 103). As the disease progresses, fibroblastic proliferation becomes more evident, and there is an associated eosinophilic and plasmacytic reaction (Fig. 104)

Nonregenerative anemia of mild to moderate degree, leukopenia, and thrombocytopenia are sometimes seen, and are occasionally marked when marrow displacement is advanced. Chemical analysis of the tissues shows a high content of cholesterol, cholesterol esters, neutral fat, and lecithin.

The disease begins in early childhood in the majority of cases, and in these instances longevity is seldom over one to four years Older persons may be similarly affected, and in some the lesions are radiosensitive and prognosis better.

Letterer-Siwe Disease. Also known as nonlipoid histiocytosis, this disease is regarded by some authors as another expression of the basic disorder underlying eosinophilic granuloma and Hand-Schüller-Christian disease. There is the same focal and diffuse proliferation of histiocytes throughout the tissues, but without lipophagocytosis. The disease is characterized clinically by fever, skin rash, progressive hypochromic anemia, and purpura, occasionally with focal destruction of bone. It occurs mostly in infants and runs a rapid and inevitably fatal course. The histologic features of the disease are similar to those illustrated under the heading of "diffuse reticulo-endotheliosis" (Figs. 268, 269). Whether this condition is inflammatory or neoplastic still remains a question.

Chemical Poisoning

Virtually the only chronic chemical poisoning that results in displacement of the bone marrow is that due to an excess of dietary fluorine. This will be mentioned in more detail in a later section (p. 183, Fig. 163).

VIII

HYPERSPLENISM

The spleen is occasionally more of a hindrance than a help. Certain forms of disordered splenic function are revealed by accelerated destruction of blood cells, by a pathological influence on blood cell formation, or both. The beneficial and often curative effect of splenectomy in a significant number of cases is ample proof that the spleen is the principal offender, the means by which the organ does harm is not so well understood. The term "hypersplenism" is recommended to encompass this particular group of splenic disorders.

PRIMARY HYPERSPLENISM

Congenital or acquired splenic dysfunctions in which no etiological agent is demonstrable are included in this category. The spleen is virtually always enlarged, although it is not necessarily palpable on physical examination With the exception of familial hemolytic jaundice, the histologic appearances of the tissue are relatively nonspecific, apart from a more or less conspicuous hyperplasia of cells of the reticulo-endothelial system Phagocytosis of blood cells by these histiocytes is not readily demonstrable in the average sections, but Doan and his associates* claim that supravital studies of the fresh splenic pulp disclose engulfed cells or cell fragments In familial hemolytic jaundice, the histologic pattern of the spleen is pathognomonic of that disease (p 127)

Hypersplenism *limited to red blood cells* is exemplified by familial hemolytic jaundice, where splenectomy nearly always effects a clinical cure (p. 127). Less specific forms of acquired hemolytic anemia are not so often splenogenous, as indicated by the relatively low percentage of patients who show improvement after removal of the spleen. Most other varieties of hemolytic anemia, such as familial erythroblastic anemia and sickle cell anemia, are unaffected by splenectomy.

Primary splenic neutropenia is characterized by *selective destruction of neutrophils* by the enlarged spleen. The bone marrow in this condition shows granulocytic hyperplasia, and removal of the spleen is followed by restoration of normal levels of granulocytes in the peripheral blood. Great care must be exercised in ruling out drug neutropenia and chronic leukopenic granulocytic leukemia, where splenectomy may do harm. Differentiation from this form of leukemia may be difficult, because the marrow in each shows granulocytic hyperplasia. I remember too well recommending that splenectomy be performed on a patient with chronic neutropenia, only to learn from the autopsy that he had had chronic granulocytic leukemia

Hypersplenism *affecting specifically the thrombocytes* characterizes idiopathic thrombocytopenic purpura While the spleen may possibly destroy thrombocytes at a rate in excess of normal, its major effect is on the megakaryocytes (p 160) These cells are present in the bone marrow in greater than normal numbers, but show a striking diminution in the production of thrombocytes. Immediately after splenectomy, thrombocyte formation begins and proceeds at an exceedingly rapid rate, and the hemorrhagic manifestations of the disease usu-

* Doan, C A, and Wright, C S Primary Congenital and Secondary Acquired Splenic Panhematopenia Blood, 1 10, 1946

Sometimes, for reasons that are not clear, skin ulcers on the legs and feet may be the presenting sign.

The urine and feces contain an excess of urobilin and urobilinogen, but there is no bile in the urine (acholuric jaundice), and the feces have a normal or slightly orange-brown color. The blood counts may be normal or show only a minor normocytic anemia. Erythrocyte configurations are variable, some conditions being characterized by spherocytosis, others by thin, flat cells and "target cells," while sickle cell anemia has its own peculiar poikilocyte. Erythrocyte fragility is also variable, being increased especially in familial hemolytic jaundice, and decreased in familial erythroblastic anemia and sickle cell anemia.

Patients with chronic hemolytic anemia are prone to develop acute exacerbations (hemoclastic crises) during which they may die, or they may recover to resume their previous state. The possible relationship of a transient lag in bone marrow regeneration to these crises will be mentioned in the discussion of familial hemolytic jaundice.

CAUSES OF HEMOLYTIC ANEMIA

Some of the causes of abnormal hemolysis can be clearly stated, while the precise mechanism by which red blood cell destruction comes about in other conditions is not definitely known. The latter group will be so designated in the following tabulation, rather than being listed on the basis of speculation

TABLE 14

I Infections
- (a) Bacterial toxins (especially *Clostridium perfringens*, streptococci), p 218
- (b) Blood parasites
  - (1) Protozoal (malaria), p 228
  - (2) Nonprotozoal (bartonellosis), p 240
- (c) Virus diseases
  - (1) Infectious mononucleosis, p. 207

II Chemical Agents, p 184

III Physical Agents
- (a) Heat (burns), p 169
- (b) Cold (activation of hemolysin) (see VI, b), p 171

IV Allergy
- (a) Favism
- (b) Baghdad anemia

V. Hemagglutinins, endogenous, *naturally occurring:*
- (a) Agglutinins alpha and beta·
  - (1) Incompatible blood

VI. Hemagglutinins, endogenous, acquired (immune body type)·
- (a) Agglutinins, anti-Rh, etc :
  - (1) Intra-group hemolytic blood transfusion reactions
  - (2) Erythroblastosis fetalis
- (b) Cold hemagglutinins, p 171
- (c) Hemolysins, endogenous, undetermined source:
  - (1) Hemolysinic anemia

VII Hypersplenism, p 113
- (a) Familial hemolytic jaundice
- *(b) Acquired hemolytic anemia (some cases)*

VIII. Mechanism Unspecified·
- (a) Familial erythroblastic anemia (thalassemia)
- (b) Sickle cell anemia
- (c) Elliptocytosis
- (d) Paroxysmal nocturnal hemoglobinuria
- (e) Malignant tumors, p 151

Certain of these conditions have already been mentioned, as indicated by the cross-references in Table 14. The others will be discussed in accordance with the classification presented on page 37, which groups them more conveniently.

HEMOLYTIC ANEMIAS PECULIAR TO INFANCY AND CHILDHOOD

Erythroblastosis Fetalis. This is a disease which occurs in late fetal life or within a few days after birth. It is nearly always due to immunization of an Rh negative mother by Rh positive red blood cells of the fetus, whereby the mother develops an anti-Rh agglutinin which passes into the fetal circulation and destroys the fetal red blood cells.

A number of factors enter into the development of the disease, other than the requirement for an Rh positive father and an Rh negative mother, accounting for the relatively low incidence (about 1 in 200 births) and the mildness of some cases The more important are (1) the male genotype; if the father is homozygous (with two dominant genes, RhRh) all offspring will be Rh positive, but if he is heterozygous (with one dominant and one recessive gene, Rhrh) half of the children will be Rh negative; (2) the requirement for fetal Rh positive erythrocytes to enter the maternal circulation, whereas the placenta

should normally screen cells and probably does in many instances, (3) the time that fetal erythrocytes pass into the mother's blood (the outlook is more serious if even a low titer of anti-Rh agglutinins is demonstrable in the mother's serum several months before term, than if a high titer is noted for the first time within the last week or so); and (4) the ability of the mother to form antibodies (some people produce them poorly)

Unless the mother has been previously immunized by transfusion of Rh positive red blood cells, the first child is invariably normal, and the second child frequently has little or no evidence of the condition. When the trend is established, subsequent pregnancies are apt to show a progressive severity, although Rh negative children from a heterozygous male may be interspersed

Other incompatibilities involving unusual blood factors, and even the major agglutinogens A and B, account for the development of erythroblastosis fetalis in a small percentage of cases. The complexities that have developed from studies on the Rh and Hr problem are far too involved to permit even mention in a book of this sort.

Several *clinical syndromes*, fetal hydrops, icterus gravis, and congenital anemia of the newborn, have been described as separate entities in the past, but have been shown to be merely variants of erythroblastosis fetalis. Edema, jaundice, and striking pallor may appear singly or in combination, and are sometimes associated with hemorrhagic manifestations The signs may be present at birth or develop within the first few days Infants who are markedly edematous or deeply jaundiced at birth are generally stillborn or die soon afterward The liver and spleen are frequently palpable and often enlarge rapidly because of extensive extramedullary hematopoiesis in these organs, a compensatory mechanism in response to the severe hemolytic anemia Respiratory embarrassment, stupor, and convulsions mark the terminal phases of the illness Much milder forms of erythroblastosis are common and would be overlooked were they not anticipated by prenatal Rh studies on the parents, anti-Rh titrations of the mothers' blood serum, and examination of the infants' blood.

The *blood* soon after birth contains nucleated red cells in excess of 10,000 per cu mm. (Fig. 106), sometimes nearing 100,000 or more (Fig. 105), in contrast to the normal maximum of about 2000, these levels tend to fall, so that nucleated red blood cells may even disappear from the circulation by the end of the first week. Anemia is generally slight the first day, but may proceed with extreme rapidity and on the fourth or fifth day erythrocyte counts may be as low as 1,000,000 per cu mm. The anemia is macrocytic and normochromic or hyperchromic. Reticulocytosis is pronounced, probably accounting for the decrease in erythrocyte fragility. Rather high leukocyte counts (after correction for nucleated red blood cells) are generally observed, often with the appearance of progranulocytes and prolymphocytes, erythrophagocytosis by monocytes is sometimes seen in the peripheral blood (Fig. 108). In severe cases, thrombocytes are reduced in number and lend to prolongation of the bleeding time, in conjunction with the hypoprothrombinemia which is frequently present Serum bilirubin values are increased and may reach high figures, owing both to hemolysis and impaired liver function

The *bone marrow* displays striking hyperplasia in the erythrocytic series, many pre-erythroblasts are present, but the vast majority of cells are in the erythroblast and normoblast stages (Fig. 109), many nucleated red blood cells are seen free in the blood sinuses At its top pitch of activity, however, the bone marrow is incapable of matching the hemolytic process, and the fetal blood-forming function of the liver and spleen is accelerated to the extent that the liver, in particular, sometimes contains much more hematopoietic than hepatic tissue. A lesser degree of blood formation goes on in the other tissues, as well.

Forewarned is forearmed *Treatment* of erythroblastosis begins in anticipation, by determining the Rh factor of all married couples during the first pregnancy of the wife Should the conditions obtain whereby erythroblastosis fetalis might develop, and should the wife have had any blood transfusions in the




past, it is well to test for the presence of anti-Rh agglutinins in the mother's blood serum every two or three weeks during the last two months of pregnancy. This is a requirement for all subsequent pregnancies. When anti-Rh agglutinins are present, one must have an adequate supply of group O, Rh negative blood available at the time of delivery (neutralized with Witebsky substance) to transfuse the inhibiting, or neutralizing substance, Rh hapten, has been described by Carter.* Its therapeutic value has not yet been fully assayed.

Nowadays, a fair number of babies are saved who previously would have died. If the baby survives the first week, its chances are good, as the hemolytic process diminishes thereafter, and with additional blood transfu-

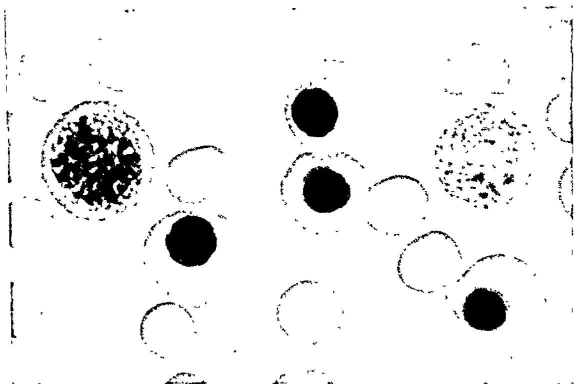


Fig 106 *Erythroblastosis Fetalis Blood, First Day.* This was the first baby of an Rh positive father and Rh negative mother who had previously received a transfusion of Rh positive blood No Rh agglutinins were demonstrable in the mother (not tested for blocking antibodies), but an anti-Rh titer of 1 8 was obtained ten days postpartum The baby's initial blood count was. hemoglobin, 12 gm.; red blood cells, 2,800,000, nucleated red blood cells, 17,300, white blood cells, 46,900 with immaturity of neutrophils to 1 progranulocyte and 1 myeloblast. An occasional proerythroblast was encountered (left), but most nucleated red blood cells were on the mature end of the scale. A metamyelocyte is seen at the right of the picture (× 2100)

infant at the first sign of the condition Even in the absence of anti-Rh agglutinins, this is good insurance, and daily blood counts should be performed on the baby during the first week Exsanguination (exchange) transfusions have been recommended, especially in severe cases, but have not been completely evaluated to date The mother must not nurse an erythroblastotic infant, as her milk contains agglutinins as well as her blood serum. An antibody,

sions as required to maintain normal erythrocyte levels, recovery may be complete in a month or two. Long-range evaluation of patients who have survived, especially as regards the central nervous system, has not yet been satisfactorily accomplished

Idiopathic Hemolytic Anemia of Infancy and Childhood. One occasionally encounters a patient presenting all of the classical signs of

* J Immunol, 61 79, 1949

hemolytic anemia as noted in the first part of this chapter, but not conforming to any specific disease entity. In making the differential diagnosis, one must remember that certain types of hemolytic anemia, notably familial hemolytic jaundice, familial erythroblastic anemia, and sickle cell anemia, may become manifest early in life, and the first manifestations may be exceedingly vague. Hemolytic anemia is occasionally the outstanding feature of Gaucher's appropriate means, whereas the idiopathic type may or may not respond to blood transfusions or splenectomy.

FAMILIAL AND RACIAL HEMOLYTIC ANEMIAS

Included in this category are familial hemolytic jaundice, elliptocytosis, familial erythroblastic anemia, and sickle cell anemia. Besides the hereditary factors, they have another



Fig 107. *Erythroblastosis Fetalis Blood, Eleventh Day* (same case as Fig 106). The level of nucleated red blood cells fell rapidly and on the eleventh day blood levels were: hemoglobin, 14 gm, red blood cells 4,480,000, nucleated red blood cells, rare, white blood cells, 23,900 with no neutrophil younger than a metamyelocyte The baby had received five blood transfusions averaging 50 cc. each in the interval The picture shows a late erythroblast (lower left), a cyte (center), and two segmented neutrophils (upper right) (× 2100)

disease and Hodgkin's disease. The infantile form of pernicious anemia may also prove confusing (p 59, Figs. 44 and 45), especially when considerable numbers of nucleated red

chiefly through a process of elimination. It is necessary that this be done well, in view of the fact that some of the other conditions under consideration can be improved or cured by

characteristic in common, an abnormality in shape of erythrocytes which is present with or without evidence of active disease.

Familial Hemolytic Jaundice (Hereditary spherocytosis, congenital hemolytic anemia, hemolytic ictero-anemia, chronic acholuric jaundice, and so on.) The disease is characterized by the overproduction of abnormally fragile erythrocytes which are destroyed at a rate in excess of normal. It is transmitted through either parent as a mendelian dominant trait, and generally

occurs in the white race, although a few cases have been described among Negroes. While the trait is present at birth, active manifestations of the disease may first appear at any age or may never become clinically evident.

The precise *pathogenesis* is not well understood It is clear that a large proportion of erythrocytes have a relatively spherical shape when compared with the usual biconcave disk, lessened resistance of these cells to hypotonic salt solution persist to some degree after splenectomy.

The *clinical course* of the disease may adopt one of several forms Some persons have no symptoms and are neither anemic nor jaundiced (latent type), with slight to moderate spherocytosis as the only evidence of the condition. Others pursue a chronic course during

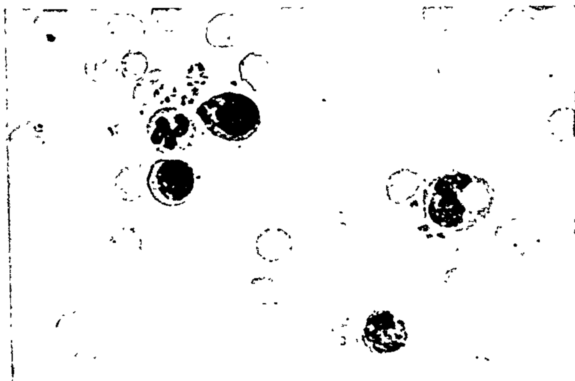


Fig 108 *Erythroblastosis Fetalis. Blood Showing Erythrophagocytosis* The baby was born of healthy parents whose Rh factor was not known, he had one sibling aged seven and healthy Icterus appeared within the first day, increased, then abated On the tenth day, pallor and splenomegaly were noted Blood examination showed hemoglobin, 3 1 gm; red blood cells, 780,000, nucleated red blood cells, 5,720, white blood cells, 26,000 with 24 6 per cent immature neutrophils He died the same day. In addition to several proerythroblasts (upper left), the field shows a monocyte which has engulfed a red blood cell (× 1000) (Slide by courtesy of Dr Tyree C Wyatt, Department of Pediatrics, Syracuse University College of Medicine.)

and consequently require the imbibition of less fluid to cause rupture of the cell membrane (in that the erythrocyte behaves as an osmometer) It is not clear whether the misshapen erythrocytes result from an inherent defect of erythropoiesis or from the action of a circulating hemolytic agent such as lysolecithin. The active role of the spleen is demonstrated by the clinical cure which follows its removal in virtually all cases, however, spherocytosis and which their complaints are minor, generally of weakness or ready fatigability, along with jaundice of varying degree and anemia which is not very marked. Because of the bilirubinemia, gallstones are apt to form, even in children, and symptoms may be primarily referable to the biliary tract. The spleen is always enlarged, but not necessarily palpable. Chronic leg ulcers, mostly around the ankles, have been described. Roentgenographic study of the

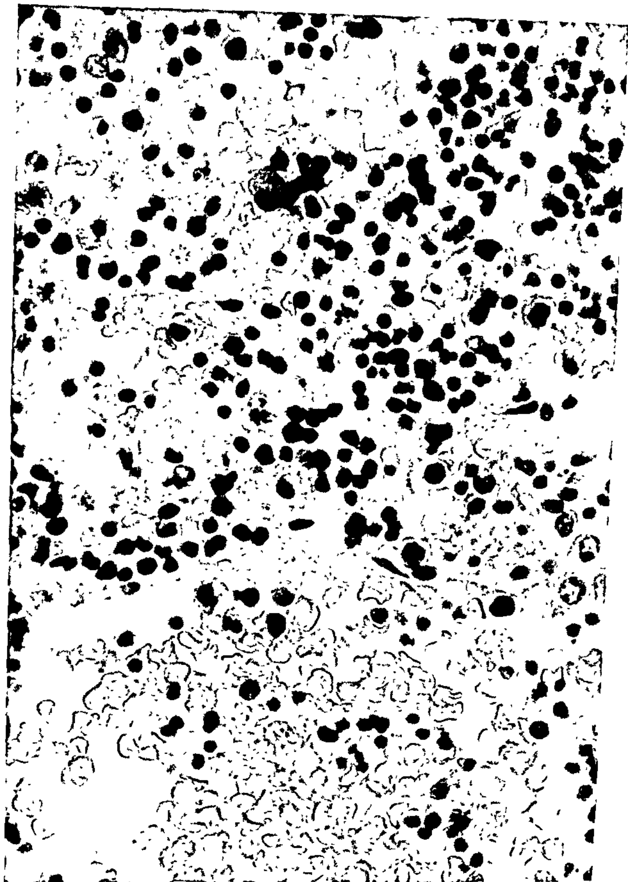


Fig 109 *Erythroblastosis Fetalis Bone Marrow Section* (same case as Fig 105) Most cells belong to the erythropoietic series They are not densely packed in the marrow space and considerable interstitial hemorrhage is evident. A large blood sinus, occupying the lower third of the field, contains many nucleated red blood cells (× 1000).

skeleton occasionally discloses rarefaction in flat bones, as well as changes in the calvarium resembling those of familial erythroblastic anemia ("hair-on-end"). Oxycephaly has sometimes been noted, as well.

Acute hemolytic crises of varying severity generally punctuate the course of chronic familial hemolytic anemia, and once in a while may be the first sign in a latent case, in the latter instance there may be confusion with acquired hemolytic anemia. The more severe crisis is frequently heralded by fever, and marked by rapidly increasing pallor, and profound weakness, often with signs of shock; jaundice may or may not deepen. There is nausea, vomiting, and abdominal pain, especially over the spleen from sudden enlargement of this organ. The patient may die during a crisis or recover from it to resume his previous status; recurring attacks during childhood often retard growth and development. The precipitating cause of these acute episodes is not known, but the fact that several members of the same household may be affected within a few days of one another is suggestive of an extrinsic factor, probably intercurrent infection.

The age at which clinical manifestations of the disease become apparent determines to some degree its seriousness When symptoms are evident in infancy or childhood, one can anticipate a more severe course with more frequent crises, if untreated, than in adults who may remain "more jaundiced than sick."

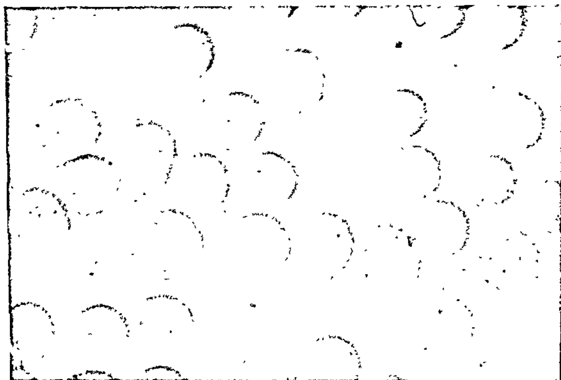


Fig 110 *Familial Hemolytic Jaundice. Blood* The patient was a ten-year-old girl who suddenly developed fever, vomiting, slight jaundice, and prostration Four days after the onset, blood examination showed hemoglobin 2 7 gm, erythrocytes 950,000 [illegible] shows most red blood cells to be spherocytic, some having slight central dimpling but none with normal concavity (× 2280)

Laboratory studies disclose the anemia, when present, to be normochromic and spherocytic (normal red blood cell volume with reduced cell diameter and consequently greater thickness). An observant technologist will often

note spherocytosis while performing the erythrocyte count (Fig. 110), for many cells will have the shape of baking-powder biscuits in contrast to the usual biconcave disks. She may also find rather marked anisocytosis because of considerable numbers of reticulocytes, which are larger than average normal erythrocytes, but there is little or no change in the circular contour of the cells. These alterations in red blood cell characters are likewise evident in the stained film, where spherocytes appear small, deeply stained, and lack central pallor, while reticulocytes are large, flat and have a bluish cast. Slight to moderate reticulocytosis is a constant feature of the latent and chronic forms of the disease, and may reach the extraordinary height of 90 to 95 per cent during the regenerative phase of acute hemolytic crises. In the early days of a crisis, however, there may be few or no reticulocytes in the peripheral blood; Dameshek and Bloom* postulated that this is due to a maturation arrest at an early level in the erythrocytic series (see also paragraph on bone marrow). Later, nucleated red blood cells, Cabot's rings, and Howell-Jolly bodies may be found in blood films as a reflection of excessive erythropoiesis.

In the average patient it is possible to demonstrate increased fragility of erythrocytes to hypotonic salt solutions, hemolysis generally beginning in concentrations of 0.65 to 0.70 per cent and being complete at about 0.40, whereas normal blood begins to hemolyze at about 0.45 per cent and is completely laked at 0.32. A considerably more informative means of performing and reporting the fragility test is described by Suess and co-workers.† Hemoglobin in the supernatant liquid from each dilution is measured in a photoelectric colorimeter and the hemolytic increments (i.e., the increase in the degree of hemolysis in successive tubes) plotted. The graphs have some re-

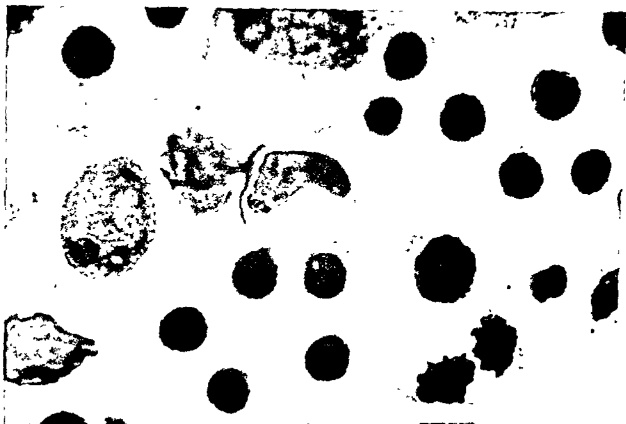


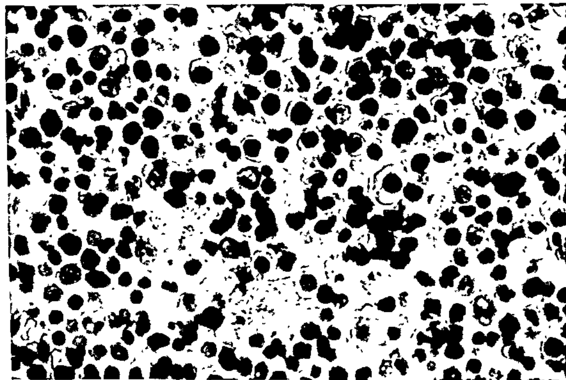
Fig. 111. *Familial Hemolytic Jaundice. Bone Marrow Smear* (same case as Fig. 110). Bone marrow aspirated on the seventh day of illness shows striking regeneration of the erythrocytic series, with most cells in the later stages of maturation. *An erythroblast in mitosis is seen in the lower right corner* (× 2280).

* Blood, 3: 1381, 1948.

† Blood, 3: 1290, 1948.

semblance to Price-Jones' curves of erythrocyte [illegible]

Opinions differ regarding the influence of reticulocytosis on fragility tests Some evidence has been presented which indicates that young red blood cells are more fragile than older ones, other data to prove that reticulocytes are normally fragile, while certain authors believe them more resistant than normal to hypotonic salt solutions. My experience tends to support the last viewpoint, based on observation of patients with an exceedingly high percentage of reticulocytes who had normal or decreased fragility tests as determined by the conventional method. For example, a boy presenting severe anemia, neutrophilic leukocytosis with immaturity, and splenomegaly had been diagnosed elsewhere as acute granulocytic leukemia, his recovery was interpreted as a spontaneous remission We observed him during a similar attack one year later Our first fragility test was reported as being normal, but the finding of a few spherocytes and a reticulocytosis of 80 per cent led us to repeat the test. This time the technologist noted a faint pink tinge to the supernatant liquid, previously overlooked, at and below the 0.70 per cent dilution. Splenectomy was followed by a rapid return of the blood picture to normal save for persistent spherocytosis, slight reticulocytosis, and hemolysis beginning at 0 52 per cent salt solution. The patient has been quite well since. I believe that Seuss' method for determining erythrocyte fragility might have clinched the diagnosis at the outset.



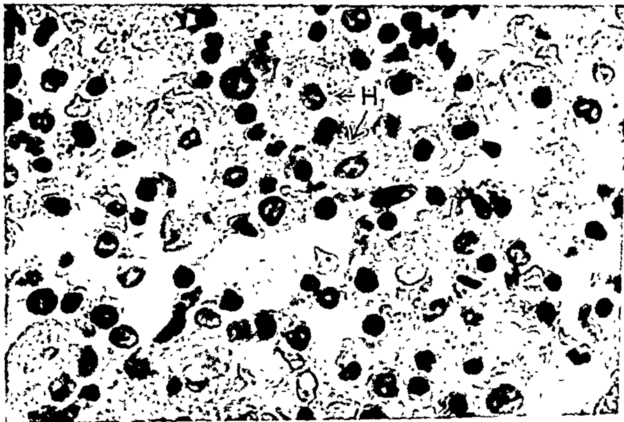
[illegible]

In the latent or chronic phases of the disease, the leukocyte count is usually normal or slightly elevated At the beginning of a crisis there is apt to be a leukopenia, but later hyperleukocytosis and even leukemoid reactions have been observed Thrombocyte levels may also be low early in a crisis, but are otherwise little disturbed.

During periods of active hemolysis the serum bilirubin is increased and the van den Bergh reaction is indirect or delayed. Urobilinogen output in the urine and feces is in excess of normal, sometimes greatly so. Bile *acids and their salts are not present in the blood* plasma or urine, hence the term "acholuric jaundice."

The *bone marrow* is invariably hyperplastic and generally contains a preponderance of erythrocyte progenitors, most of which are in the late erythroblastic and normoblastic stages (Fig. 111 and 112). Cells of the granulocytic series, while relatively diminished in proportion to the erythrocytic series, are actually present in normal or increased numbers. Megakaryocytes show no significant deviation from the normal.

Dameshek and Bloom* described an abnormality in erythropoiesis, regarded as a maturation arrest, occurring during the crisis in association with accelerated hemolysis and contributing to the rapid reduction in red blood cells. They found most cells of the erythrocytic series in early stages of development at the height of the crisis. Five days later, normoblastic erythropoiesis was observed, "*indicating* that the arrested maturation had run its course." I noted a similar marrow picture in a child who died on the fourth day of an acute hemolytic crisis (Fig. 113), but interpreted it somewhat *differently*. It seemed logical to assume that the severe hemolytic episode created a sudden demand for erythrocytes, and that the marrow responded with a wave of their progenitors. An early marrow sample would show the components of this cell wave in predominantly immature stages, not necessarily "arrested" there, whereas a later biopsy would permit



The marrow specimen was removed at autopsy from a
The marrow is not so densely packed with nucleated red

(× 1000)

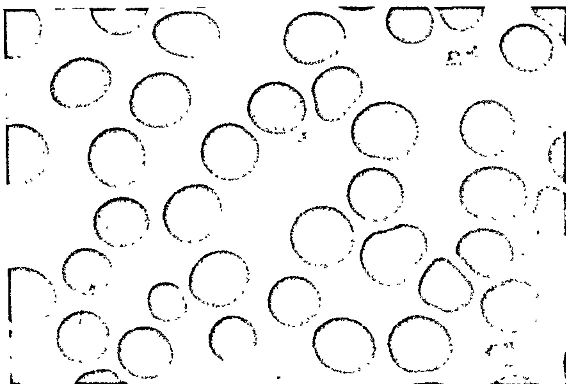
* Blood, 3 1381, 1948.

time for them to sweep through their developmental sequence.

The marrow in some cases contains considerable numbers of histiocytes showing rather marked erythrophagocytosis. I have seen this only in patients who had received blood transfusions, and have never been sure whether the engulfed red cells belonged to patient or donor, but the latter is more likely.

There is a paucity of nucleated cells, the most conspicuous ones lining blood sinuses.

Splenectomy is the *treatment* of choice, even though the operative mortality is between 3 and 4 per cent. A lasting cure almost invariably follows in those who survive the operation. Great care must be taken to find and remove any accessory spleens, as the disease will recur if this is overlooked It is also well to investi-



tomy disclosed hemoglobin 12 5 gm ; erythrocytes 4,500,000, reticulocytes 3 per cent, leukocytes 12,700 The blood film pictured here shows persistence of spherocytes, only a few cells having central pallor, erythrocyte fragility remained slightly above normal (× 2280)

The *spleen* in familial hemolytic jaundice deserves mention, because of its importance in the pathogenesis of the disease, and because its microscopic appearances are virtually pathognomonic of the disease. The organ is always enlarged from two to ten times normal size, usually having a thin smooth capsule free of adhesions, and a homogeneous dark red pulp. Histologically the pulp is literally stuffed with erythrocytes, while the sinuses are relatively empty, follicles are small and widely spaced.

gate the biliary tract at the time of operation because of the fairly high incidence of cholelithiasis in this condition.

Leukocytosis and thrombocytosis occur immediately after the operation and may persist for some time. The increase in thrombocytes may predispose to thrombotic phenomena. Evidences of abnormal hemolysis and accelerated erythropoiesis disappear promptly, and normal red blood cell levels are restored within a few weeks. Spherocytosis and increased

erythrocyte fragility generally persist, although at a lower level than before, and the reticulocyte count often remains a little higher than normal (Fig. 114).

Elliptocytosis (Ovalocytosis). This is a hereditary anomaly in which 25 per cent or more of erythrocytes have an oval, sausage, or rod shape (Fig. 115). It is transmitted as a true mendelian dominant trait. Similar cells are found in a large percentage of normal people, but in very small numbers, and they constitute a common form of poikilocytosis in a wide variety of anemias.

It is perhaps wrong to include the condition under the hemolytic anemias, as most observers believe that there is no relationship between the two Cooley* pointed out, however, that about 15 per cent of 246 persons with the anomaly were anemic, the anemia being mostly of hemolytic type. In the same report, Cooley described a different form of hereditary anemia in which elliptocytosis was pronounced in the active phase, but disappeared during convalescence.

In the usual asymptomatic person with elliptocytosis, the anomalous erythrocytes have precisely the same properties as normal ones, except for a lesser tendency toward rouleaux formation; consequently the sedimentation rate is slowed. Leukocytes and thrombocytes are quite normal.

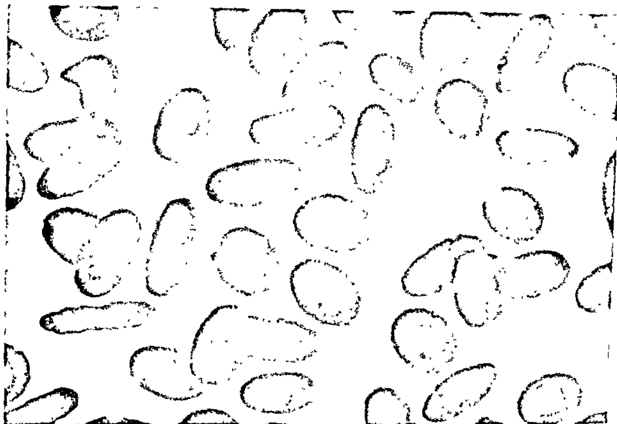


Fig. 115 *Elliptocytosis* (Ovalocytosis) *Blood* This person, two of four siblings, and the mother had elliptical erythrocytes, comprising nearly 100 per cent of all red blood cells. Members of earlier generations were not available for study. The patient had a mild hypochromic microcytic anemia and a slight reticulocytosis, but others in the family showed normal blood levels; it was not evident whether there was a relationship between the anemia and the anomaly (× 2280)

The bone marrow was not hyperplastic in the several patients examined by Wyandt and co-workers,† nucleated red blood cells and most reticulocytes were round, so that the abnormal shape is a function of the mature cell.

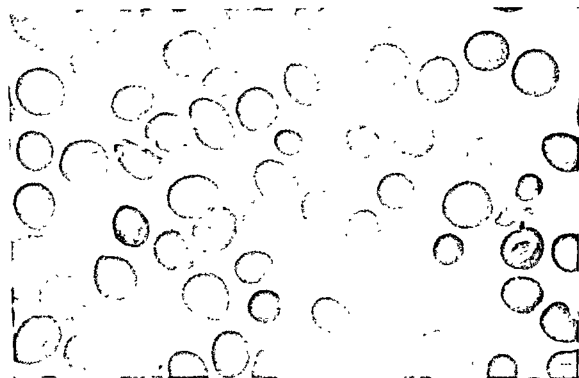
Familial Erythroblastic Anemia (Thalassemia, Mediterranean anemia, Cooley's anemia) Recent studies on recognition of the mild and "carrier" forms of familial erythroblastic anemia have made it clear that the disease is transmitted on the basis of a dominant heredi-

* Am J Dis Child., *64* 190, 1942

† Ann Int Med, *68* 1043, 1941

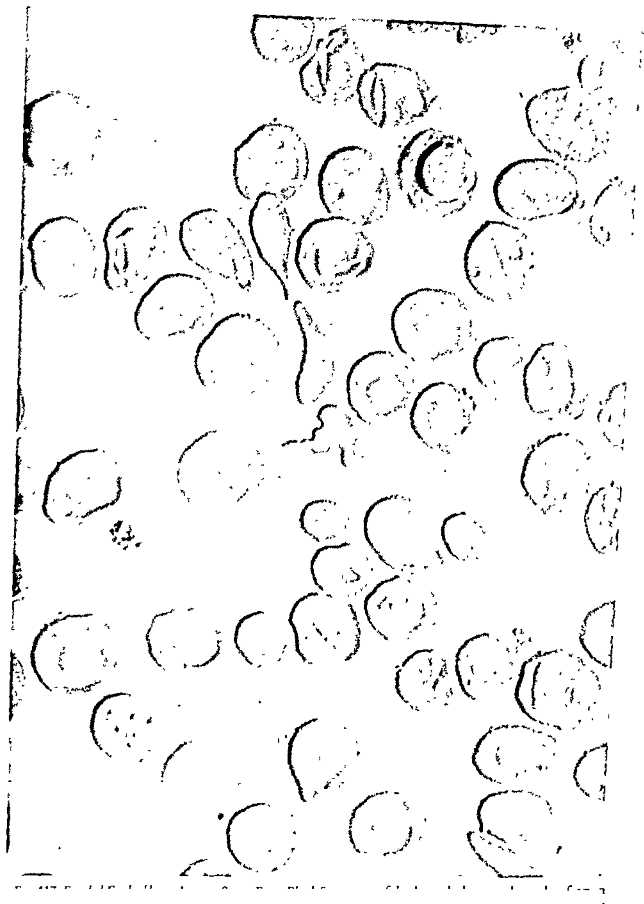
tary factor. In addition to the familial trait, nearly all cases are restricted to persons of Italian, Greek, or Syrian ancestry, a few exceptions having been reported. The nature of the inherited defect in formation of red blood cells is not understood. Hemolysis probably plays some part in the production of anemia, but not likely a fundamental one.

Clinical manifestations are usually absent in persons with the *trait* or the *mild form* of the disease. Their general appearance is normal, and at most they may have slight pallor and a palpable spleen. A suggestion of osteoporosis has been noted on roentgenographic study in relatively few patients.



The *severe form* becomes evident during infancy or childhood, usually with pallor as the first sign, and often a subicteric tinge A progressive protuberance of the abdomen calls attention to enlargement of the spleen and liver, owing in part to extramedullary hematopoiesis. Lymph nodes may also be slightly enlarged for the same reason. Changes in the skeleton become more and more pronounced as the disease advances, resulting from an extreme hyperplasia of the marrow. In addition to generalized osteoporosis, bones of the skull are widened; roentgenograms of the calvarium often disclose perpendicular striations between the tables, giving the "hair-on-end" appearance Bony changes in the skull contribute to the typical mongoloid facies. Enlargement of the cardiac silhouette, hemic murmurs, dyspnea on exertion, weakness, gastro-intestinal complaints, and bouts of unexplained fever are also commonly encountered. Edema, serous effusions, and hemorrhagic phenomena are late manifestations.

Examination of the *blood* in *mild cases* may show a slight to moderate anemia, a normal erythrocyte count, or even a minor polycythemia. Hemoglobin and volume of packed



increased resistance of erythrocytes to hypotonic salt solutions. The bas relief emphasizes the flatness of red blood cells, along with many target cells and tailed poikilocytes, a nucleated red blood cell is seen in the upper right (× 2100)



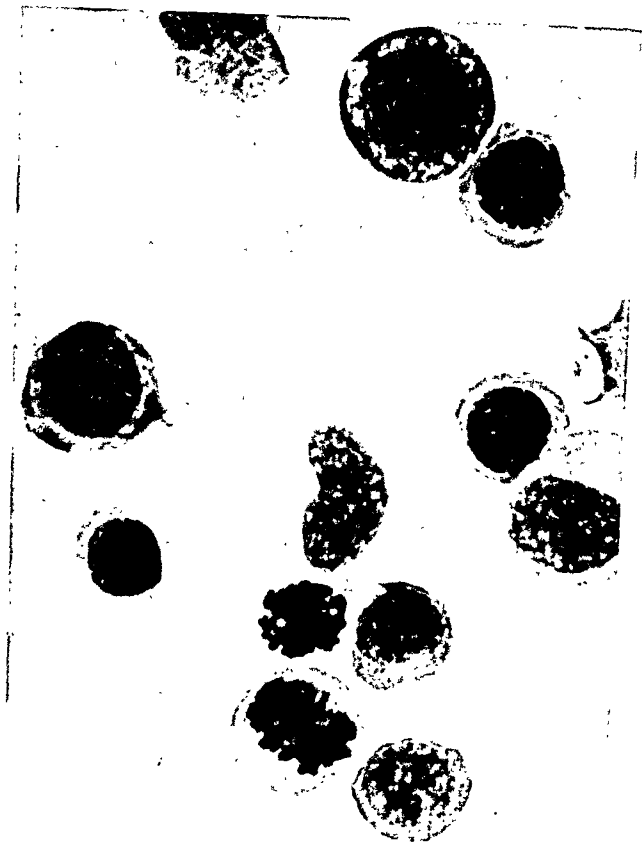


Fig 119 *Familial Erythroblastic Anemia, Severe Form Bone Marrow Smear.* This is the case of an adolescent male of Italian parentage who was severely anemic and received five blood transfusions in quick succession prior to the sternal aspiration, accounting for the relatively normal erythrocytes seen in the smear All but two of the nucleated cells in the field (center and right center, a metamyelocyte and myelocyte respectively) are early or intermediate erythroblasts, two (lower center) in mitosis (2280)



Fig 120 *Familial Erythroblastic Anemia, Severe Form Bone Marrow Section* (same case as Figs 117 and 118) The myeloid cavity is completely filled with hematopoietic tissue, most cells belonging to the erythrocytic series They vary in maturity from the large proerythroblasts to small normoblasts with compact nuclei, but intermediate erythroblasts predominate The section is taken through a vertebral body and shows complete loss of bone cortex over this sector, with marrow impinging on the periosteum (× 1000)

red blood cells are appreciably below normal, however, and morphologic changes in erythrocytes are striking, even in the absence of anemia (Fig. 116). Anisocytosis with hypochromic macrocytes is almost always present. "Target cells" are generally but not necessarily seen, as well as poikilocytosis and basophilic stippling. There is unusual resistance of erythrocytes to hypotonic salt solutions. Smith* noted the comparison with normal fragility to show most evident differences in dilutions of 0.375, 0.35, and 0.325 per cent of sodium chloride solution, and recommended that these three concentrations be used as a screening test.

Anemia is usually pronounced in *severe cases, and is hypochromic and microcytic in* type, despite the fact that many of the erythrocytes have a diameter of 15 μ. These large flat cells, poor in hemoglobin, are most characteristic of the disease (Fig 117); having likened the spherocytes of familial hemolytic jaundice to baking-powder biscuits, I would describe the red cells of familial erythroblastic anemia as griddle cakes Extremely small erythrocytes are also found, as well as a variety of poikilocytes and a good many target cells. Nucleated red cells are present in the circulating blood, and may exceed 100,000 per cu.mm. (Fig. 118); most are late erythroblasts and normoblasts, but proerythroblasts are often found Removal of the spleen may precipitate a tremendous erythroblastosis, occasionally reaching 500,000 per cu.mm.

The *bone marrow* is hyperplastic and shows inversion of the erythrogranulocytic ratio, even in mild cases, and in the severe type, erythropoietic activity reaches an extreme degree (Figs, 119 and 120). Large numbers of proerythroblasts are present, as well as undifferentiated cells, but the maturation sequence in the erythrocytic series is undisturbed There may be an associated hyperplasia of the granulocytic series, but even so, these cells are in the background. Megakaryocytes display no significant change In common with the tissues at large, the marrow contains deposits of iron-containing and iron-free pigment, generally engulfed by macrophages, foam cells (lipid-bearing macrophages) are also present, sometimes in such numbers that confusion with the so-called storage diseases might arise. Areas of fibrosis are occasionally encountered, usually in association with foci of heavy pigment deposits. The cortical bone is thinned and often lost, so that the marrow frequently lies in apposition to periosteum; trabeculae of flat bones display proliferative changes.

Treatment is restricted to transfusions of whole blood as indicated by the degree of anemia. Whereas administration of iron fills the need in most hypochromic anemias, it is quite ineffectual in familial erythroblastic anemia. This is the basis of the suggestion that the disease may be due to an inherent defect in iron metabolism.

The *outlook* is directly proportional to the age at which the disease becomes manifest. Those patients who have symptoms in childhood rarely reach adult life At the other end of the scale, persons with the mild or carrier forms are rarely aware of their disease, and their life is seldom shortened thereby.

Sickle Cell Anemia. A particular kind of poikilocytosis, variously termed sicklocytosis, drepanocytosis, or meniscocytosis, is inherited as a mendelian dominant characteristic almost exclusively by members of the Negro race. It appears in several forms, (1) the sickling trait, (2) latent sickle cell anemia, and (3) active sickle cell anemia. The factor or factors which determine the type in a person are not known. It is probable that the only difference between the three is one of degree, but the question has been raised as to whether people with the trait are more apt to develop active sickle cell anemia than those in whom the trait is not demonstrable.

An inherent defect in erythropoiesis results in red cells whose shape is potentially deformed, the actual deformity being brought about by a decrease in oxygen tension. This can be demonstrated in vitro and furnishes the basis for identification of the condition. In a relatively anoxic suspension, erythrocytes assume a variety of shapes, of which sickled or crescentic forms are the most characteristic, however, other forms have been likened to oat grains, holly leaves or fish fins (Figs. 121

* Am J Dis Child, 75 505, 1948

and 123), all having in common two or more sharp points in their contour. One of the more reliable methods of producing the deformity in vitro is to place a rubber band tightly about the person's finger for five minutes before drawing blood from the tip, then placing a drop on a slide thinly coated with brilliant cresyl blue as for a supravital preparation, a cover slip is quickly applied and the edges sealed with petroleum jelly The slide is incubated at 37° C. and examined under the microscope at frequent intervals; it should not be reported as normal unless sickling has failed to appear after standing at least twenty-four hours The phenomenon can be hastened by adding a loopful from a culture of an oxyphilic bacterium to the drop of blood. Alkalinity will inhibit sickling.

Another means of identifying sicklemia employs the erythrocyte sedimentation rate, and is based on the fact that, when oxygen saturation is restored, the sickling process is reversible Sickled red cells do not form rouleaux, and consequently sediment more slowly than normal. In performing the test, one contrasts the sedimentation rate of blood exposed to



Fig 121 *Sickle Cell Anemia Blood* A young adult Negro was admitted to the hospital complaining of severe pain in the right lower quadrant of his abdomen. He had fever, nausea, vomiting, and neutrophilic leukocytosis Further examination of the blood disclosed marked normocytic, normochromic anemia, with the appearance of sickled and nucleated red blood cells in the stained blood film The cell in the upper left corner of the picture is especially typical of the disease In sealed wet preparations, nearly all erythrocytes showed distortions similar to those pictured in Figure 123 (× 2280)

oxygen and carbon dioxide respectively, a positive result being indicated by a significant difference in the rate of fall during one hour. T[illegible]
b[illegible]
n[illegible]
parameter" of over 27 mm. difference in the hour was not a reliable indication of sickle cell anemia

The *sickling trait* has been noted in about 7 or 8 per cent of Negroes, although the incidence in one series was reported as high as 14 per cent Those who possess the trait are quite healthy, and their blood levels are unchanged from the normal They may become anemic from some other cause, however, and *it is dangerous to assume that an anemic person with sicklemia is necessarily suffering from sickle cell anemia* I made this mistake in the case of a Negress who had pernicious anemia and sicklemia. *She had received a small amount of folic acid prior to bone marrow biopsy, enough to alter the marrow picture somewhat, but not enough to induce a remission. A second biopsy, after about six weeks without any medication, set matters straight.*

Latent sickle cell anemia denotes the condition in which minor changes in the blood may be noted, along with few or no complaints or physical findings. Active phases of the disease may be interrupted by more or less extended latent periods; if the patient is first seen at this time, the diagnosis is oftimes difficult to make.

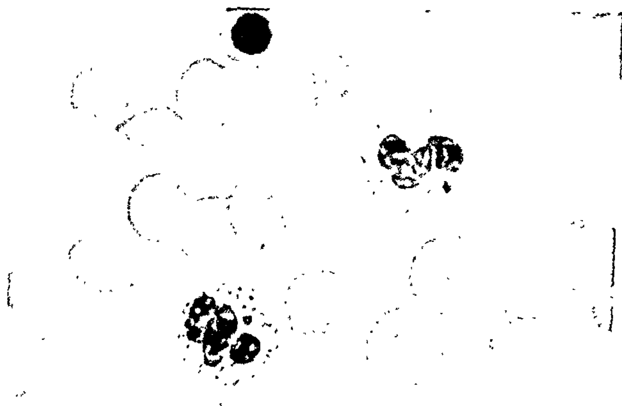


Fig 122 Sickle Cell Anemia Blood The patient was a middle-aged Negress who rapidly developed weakness and dyspnea on exertion She gave a history of many such episodes in the past, from which she recovered spontaneously. Scars of recurrent skin ulcers were noted above each ankle She was markedly anemic The stained blood film contained target cells and nucleated red cells, but no sickling was evident beyond an occasional bipolar erythrocyte (upper left) (2100)

Active sickle cell anemia refers to the clinically evident disease which may have its onset in infancy or childhood, or may be delayed for a considerable number of years Many patients give a history of recurring illness with interval periods of relatively good health, the exacerbations representing hemolytic crises. Others

* Blood, 4 179, 1949

will remain rather severely anemic between attacks owing to a more sustained hemolytic process.

The *symptoms* are exceedingly variable, to the extent that the disease has been confused with appendicitis, typhoid fever, hepatitis, rheumatic fever, acute polyarthritis, osteomyelitis, and meningitis Weakness and easy fatigability are rather constant complaints. A subicteric tint and mucosal pallor are frequently observed. These become more marked during an exacerbation, usually associated with been reported in a few cases. Chronic leg ulcers occur much more frequently than in other forms of hemolytic anemia, although they seldom develop during childhood. Roentgenographic study of the skeleton discloses changes akin to those described in familial erythroblastic anemia, except that vertical striation in the skull is not so common; narrowing or obliteration of the medullary cavity may be found in long bones, owing to associated osteosclerosis Many of the symptoms and signs have been laid to capillary

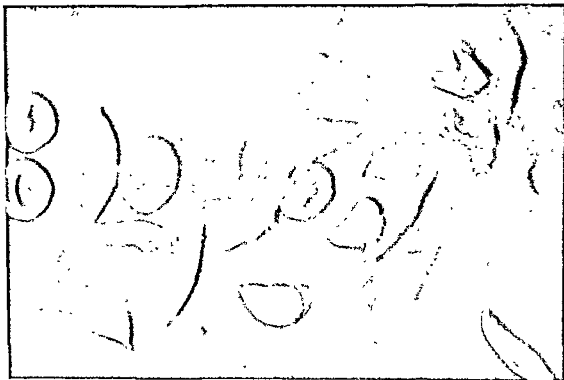


Fig 123 *Sickle Cell Anemia Blood, Wet Sealed Preparation* (same case as Fig 122) About 80 per cent of erythrocytes have adopted sickled and other bizarre multipointed forms (× 2100)

fever and pain referred to the abdomen or extremities, a variety of neurologic disturbances have been described, and retinal vessels are commonly dilated and tortuous The heart is generally enlarged, and hemic murmurs are heard Chest signs sometimes simulate pneumonia Splenomegaly may be prominent early in the disease, especially in children, but recedes with successive attacks, to the point that the organ is occasionally reduced to a small fibrous mass in those who survive until later life The liver is usually enlarged Priapism has stasis and thrombosis in the various tissues, and the resultant anoxia. Just how this condition comes about is poorly understood at the present time.

Examination of the *blood* during the latent phase generally discloses a mild normocytic and normochromic anemia, whereas in periods of increased activity the anemia is moderate to severe and may be either slightly macrocytic or microcytic. Reticulocytosis is present in proportion to the severity of the hemolytic process, averaging around 25 per cent, often

much higher; the reticulocytes are rarely poikilocytic. Nucleated red blood cells are commonly found during a crisis, as are stippled cells and Howell-Jolly bodies. In the dried, stained film, sickle cells are not usually conspicuous, and there is no correlation between their number and the severity of the disease (Fig. 122); the procedure for developing the abnormality in wet preparations was mentioned earlier (Fig. 123). Many "target cells" may be present (Fig. 122). Leukocytosis is regularly found, and a leukemoid reaction may develop during an acute hemolytic phase, often associated with a significant increase in thrombocytes. Erythrocytes are more resistant than normal to hypotonic salt solutions, hemolysis beginning about 0.34 per cent sodium chloride and being complete in the neighborhood of 0.12 per cent. The slowness of the erythrocyte sedimentation rate has already been discussed.

Bilirubin in the serum, and urobilinogen in the urine and feces are increased in direct proportion to the rate of hemolysis just prior to the examinations.

The *marrow* spaces of all bones, with the possible exception of the hands and feet, are filled to brimming with hematopoietic tissue, the bulk of cells belonging to the erythrocytic series (Fig. 124 and 125). Erythropoiesis is normoblastic in type, and normoblasts only rarely show distortions in contour comparable to those of erythrocytes. In formol-fixed specimens, sickling of red cells is striking, much less so when the tissue has been fixed in Zenker's solution. Cells of the granulocytic series are relatively reduced but actually increased in numbers, especially eosinophils, and are qualitatively normal. The same may be said for megakaryocytes. In cases of long standing, deposits of hemosiderin are abundant, most pigment engulfed by histiocytes. Fibrous patches and areas of new bone formation occur particularly in marrow of long

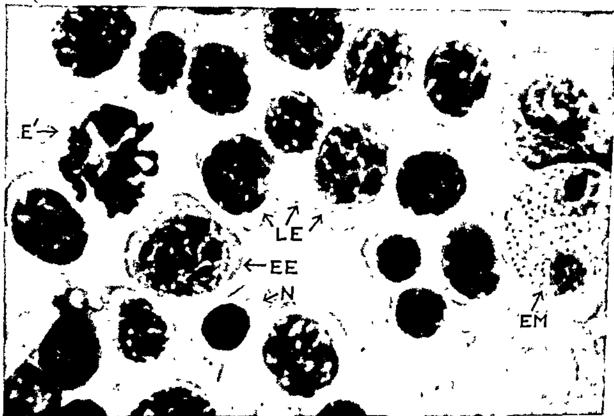


Fig. 124. *Sickle Cell Anemia. Bone Marrow Smear* (same case as Figs. 122 and 123). The aspirate was composed of almost completely erythropoietic marrow, with most cells in the erythroblastic and normoblastic stages of development (EE, early erythroblast, LE, late erythroblast, E′, erythroblast in mitosis, N, normoblast, EM, eosinophil, mature) (× 2280)

bones and probably mark the site of intramedullary thromboses or hemorrhages.

The *spleen* presents a characteristic lesion, described by Rich,* of a moat of blood around malpighian corpuscles Infarcts are common, as well as focal hemorrhages in the pulp, the latter being followed by the formation of siderocalcific fibrous nodules (Gamna-Gandy bodies) This repeated scarring accounts for the progressive contracture of the organ.

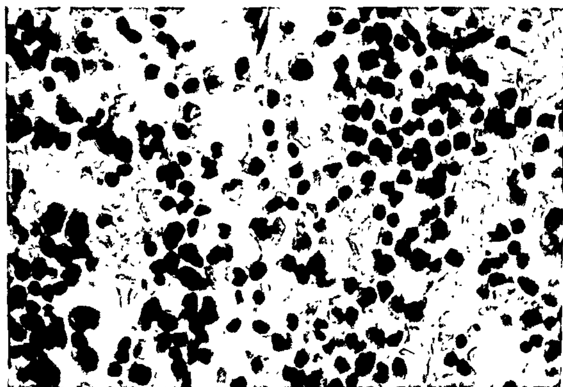


Fig. 125 *Sickle Cell Anemia Bone Marrow Section* (same case as Figs 122, 123, and 124) Section through an aspirated fragment of marrow shows all fat replaced by hematopoietic tissue, most cells being intermediate and late forms of red blood cell progenitors, as indicated by their relatively small size and compact nuclei (× 1000).

Similar lesions may be found in other tissues, especially lungs and kidneys.

Active *treatment* is limited to transfusions of fresh whole blood as indicated Oxygen inhalation has been recommended, to be given during crises, especially when pulmonary complications are evident The results of splenectomy do not justify the risk entailed.

The *outlook* is unfavorable and varies to some extent with the age at which the disease becomes manifest, the younger the patient the poorer the prognosis. Some who survive for a good many years are handicapped by chronic debility and recurrent acute illness.

* Bull Johns Hopkins Hosp , 43 398, 1928

ACQUIRED IDIOPATHIC HEMOLYTIC ANEMIAS

After one eliminates any possibility of hemolytic anemia being inherited or secondary to some recognizable cause, there still remains a group of patients in whom anemia is obviously due to abnormal destruction of red blood cells. In some of these cases, endogenous hemolysins in high titer have been demonstrated in the patients' blood.† They have certain properties of immune bodies (inactivation by heat, reactivation by complement, increased activity following short exposure to cold, decreased activity from long chilling, positive Ehrlich-Morgenroth reaction).

It is not altogether clear whether isohemolysins are the sole cause of anemia in

† Dameshek, Schwartz, and others Medicine, 19 231, 1940

these patients, or whether they are generated by an *initial* hemolytic episode and merely serve to augment and continue the process. In favor of the latter view, I know of at least one case in which the hemolysin titer fell rapidly after splenectomy, concomitant with recovery of the patient. At any rate, an effort should be made to identify isohemolysins when no other cause for the anemia can be found.

weakness, dyspnea, palpitation, lethargy, and even coma and paralysis. Mild to moderate icterus appears. Hemic murmurs are nearly always heard, and the cardiac silhouette is frequently widened. The spleen is regularly enlarged, although it is not necessarily palpable, and liver enlargement has been noted in about one-third of the cases.

Examination of the *blood* shows anemia

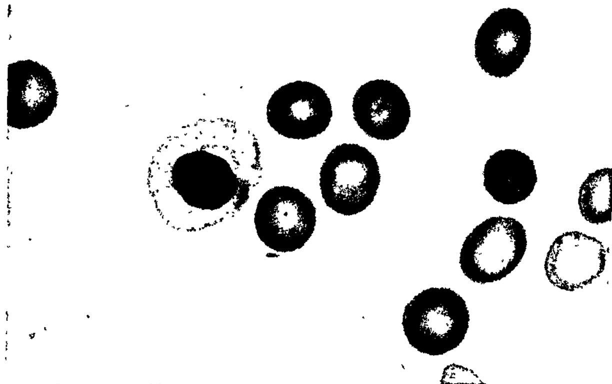


Fig 126 *Acute Acquired Idiopathic Hemolytic Anemia. Blood, Early Phase* A white male aged twenty-three years, previously healthy and with no family history of anemia, suddenly developed fever, back pain, anorexia, and vomiting, this was followed by pallor, slight jaundice, and prostration The first blood study disclosed hemoglobin, 7 gm; erythrocytes, 2,570,000, reticulocytes, 2 per cent, a slight tendency toward spherocytosis, and erythrocyte fragility barely over normal limits, leukocytes, 12,300, with a few immature neutrophils A late erythroblast is seen in the left center, and a spherocyte to the right of center (× 2280).

Acute Acquired Idiopathic Hemolytic Anemia (Lederer's anemia). The disease may occur at any age, mostly before the twentieth year, although older persons are occasionally affected There is no predilection for sex or race.

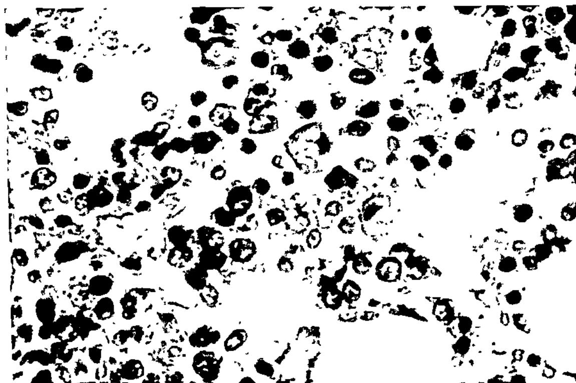
The onset is generally sudden or fairly so. Early symptoms are fever, gastro-intestinal disturbances, and generalized aches and pains, so that the provisional diagnosis of "intestinal flu" has sometimes been made The rapid development of anemia is manifest by pallor,

developing at an amazing rate in the more severe cases, the erythrocyte count reaching 1,000,000 per cu mm. or less within a few days' time Depending to some extent on the degree of spherocytosis and reticulocytosis, the anemia may be normocytic, microcytic or macrocytic. Erythrocytes may vary greatly in diameter, but there is little or no distortion in their contour. A high percentage of reticulocytes (Fig. 128) and many nucleated red cells (Fig. 129) are characteristically found in the

peripheral blood. The fragility of erythrocytes in hypotonic salt solutions may be normal or increased, in direct proportion to the degree of spherocytosis.

The leukocyte count is almost constantly elevated (Fig 129), and levels as high as 132,000 per cu mm. have been reported, with a marked "shift to the left" in the neutrophil series Relatively normal counts, at least early

The *bone marrow* rapidly undergoes hyperplasia (Fig. 127, 130, and 131), with cells of the erythrocytic series predominating. Erythropoiesis is normoblastic in type. Erythrophagocytosis may be conspicuous (Fig. 127), and hemosiderin deposits are frequently found in cells of the reticulo-endothelial system. The granulocytic series shares the hyperplasia (Fig. 130), but to a much lesser degree.



[illegible] erythroblasts and normoblasts There is an abundance of histiocytes engorged with red blood cells, the patient had a blood transfusion prior to the biopsy, so the partly destroyed erythrocytes might belong to the patient, the donor, or both (× 1000)

in the disease (Fig. 126), and even leukopenia, have been observed In children there may be a marked lymphocytosis with some immaturity, although this is seldom seen. Thrombocyte levels are relatively undisturbed.

In common with all hemolytic anemias, the serum bilirubin is elevated, and urinary and fecal urobilinogen output markedly increased. In severe cases, hemoglobinuria may be an early evidence of blood destruction, and on a few occasions this has been followed by anuria and uremia.

Treatment with blood transfusions in some instances will effect a cure. One must insure against hemolysins in the patients' blood by incubating for one hour a suspension of donor's cells in patient's serum. Blood should always be given slowly Should the patient fail to respond to transfusions, splenectomy must be considered. While the curative effect of splenectomy is not nearly so uniform as in familial hemolytic jaundice, and the operative mortality is considerably higher, dramatic recovery in a number of cases has followed the

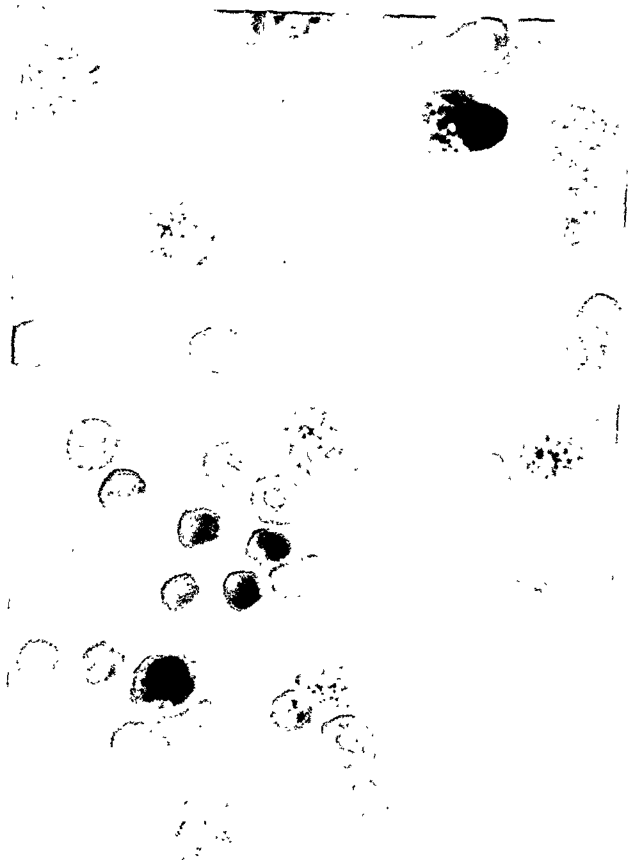


Fig 128 *Acute Acquired Idiopathic Hemolytic Anemia Blood, Later Phase, Supravital Stain* (Same case as Figs. 126 and 127) The cytoplasmic skein is clearly seen in reticulocytes and normoblasts, reticulocytes at this time comprised 30 per cent of total non-nucleated cells Spherocytes are distinguished by their narrow diameter and dark coloration (film counterstained by Wright's technic) (× 2280)

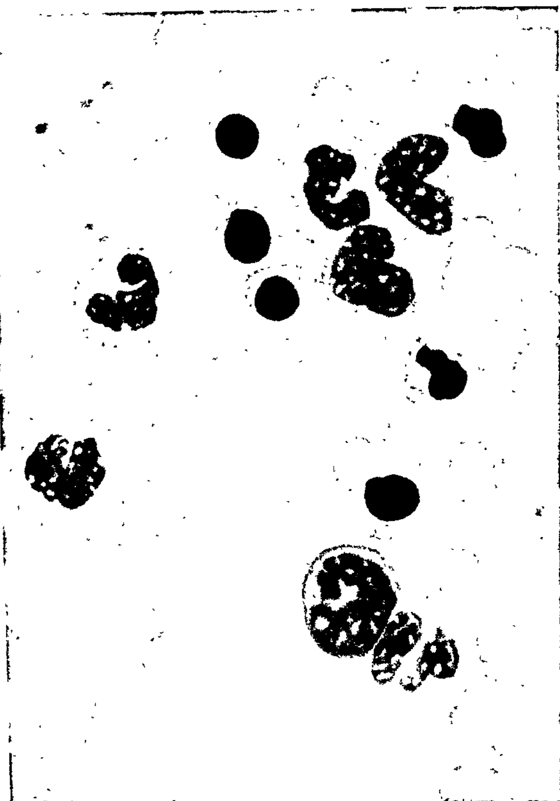


Fig 129 *Acute Acquired Idiopathic Hemolytic Anemia Blood, Still Later* (same case as Figs 126, 127, and 128). The peripheral blood is flooded with nucleated red cells and neutrophilic leukocytes, with a shift to the left amongst the neutrophils The small, dark orange cells are spherocytes, the larger paler forms with a bluish cast reticulocytes (× 2280)



Fig. 130 *Acute Acquired Idiopathic Hemolytic Anemia Bone Marrow Smear* (same case as Figs 126–129) Most cells belong to the erythrocytic series and are found in all stages of development from the proerythroblast (lower right, partially shown) to the more prevalent later forms with compact nuclei. The outnumbered cells of the granulocytic series are still present in considerable numbers (near lower, left, and top margins) (× 2280)

operation. Other patients proceed to die, irrespective of the form of treatment.

Chronic Acquired Idiopathic Hemolytic Anemia. This condition bears a superficial resemblance to chronic familial hemolytic jaundice in its clinical manifestations, including a tendency to develop acute or subacute exacerbations, but differs in certain fundamental respects It has no familial association and generally occurs in an older age group. The spleen is larger as a rule, owing more to reticulo-endothelial hyperplasia than engorgement of the pulp with erythrocytes. While removal of the spleen is frequently followed by improvement in the clinical status of the patient, it is never so spectacular, and splenectomy often fails to alter the course of the disease. Microspherocytosis has been observed in a relatively small percentage of patients, these being the ones that do best after splenectomy. The anemia in the others is apt to be macrocytic, without alteration in fragility of erythrocytes.

The bone marrow picture is quite the same as we find in the familial disease. Extramedullary hematopoiesis is commonly observed. I have performed autopsies on a few such patients who were refractory to all forms of treatment and obtained no clues as to the pathogenesis of the condition

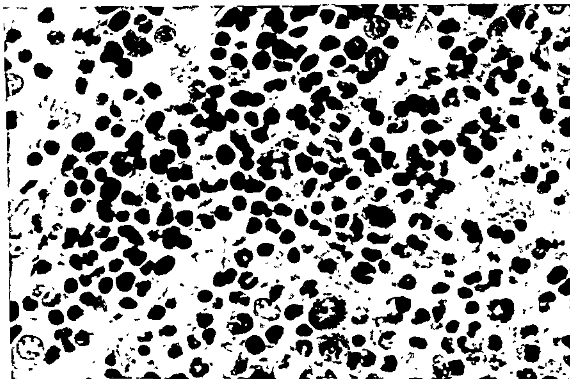


Fig 131 *Acute Acquired Idiopathic Hemolytic Anemia Bone Marrow Section, Autopsy* (same case as Figs 126–130) Even though considerable spherocytosis was present, splenectomy failed to induce a remission, and the patient died

PAROXYSMAL HEMOGLOBINURIA

The term "hemoglobinuria" denotes the presence of free hemoglobin in the urine in the absence of intact red blood cells, being thereby quite different from hematuria. It follows intravascular hemolysis of such magnitude that the reticulo-endothelial system is unable to convert liberated hemoglobin to bilirubin before the renal threshold for hemoglobin has been exceeded. The color imparted to the urine varies from light claret to black,

is directly proportional to the amount of pigment present in the urine, and reflects the intensity of the hemolytic process going on within the blood vessels.

Most of the conditions in which hemoglobinuria occurs have been considered elsewhere in the book. Those to be mentioned here are ones in which hemoglobinuria is the outstanding feature.

March Hemoglobinuria. Certain people will pass varying amounts of oxyhemoglobin in their urine after strenuous exercise, occasionally enough to color the urine black, but mostly it is pink or light red. The cause of the intravascular hemolysis leading to hemoglobinuria is not known beyond its relation to exertion.

The condition is harmless, symptomless, and unassociated with demonstrable changes in the blood or blood-forming organs. It is mentioned here to be distinguished from more serious forms of hemoglobinuria, notably cold hemoglobinuria and paroxysmal nocturnal hemoglobinuria, by employing the Donath-Landsteiner reaction and the heat resistance test respectively.

Cold Hemoglobinuria The activation of hemolysins by exposure to cold will be considered in Chapter XII, page 171.

Paroxysmal Nocturnal Hemoglobinuria (Marchiafava-Micheli syndrome). This is a rare form of chronic hemolytic anemia characterized by continuous intravascular destruction which is intensified during sleep, and is followed by the passage of dark urine after waking Ham* concluded that the fundamental defect lies in the erythrocytes, which are abnormally subject to breakdown during periods of increased acidity of the blood, a serum factor may also be involved.

The symptoms are those of any chronic hemolytic anemia, and malaise and various aches or pains are especially troublesome following sleep. Intercurrent infections tend to exaggerate the hemolytic process. The patients appear pallid and slightly to moderately icteric The spleen and liver may be enlarged, but not markedly so.

Anemia is generally rather severe, and may be either normocytic or macrocytic, with a color index around unity. Reticulocytosis of 10 to 25 per cent is usual, with nucleated red cells often being found in blood films. Spherocytes are seldom seen, and the erythrocyte resistance to hypotonic salt solutions or saponin is normal. The leukocyte and thrombocyte counts may be normal, but are more frequently reduced, leukopenia being due to an absolute decrease in neutrophils. Serum bilirubin is elevated, and free hemoglobin can be demonstrated in the plasma on spectroscopic examination, even in the absence of frank hemoglobinuria.

The so-called "heat resistance" test, which probably depends on increased acid production rather than the action of heat, is simple and fairly reliable. About 5 ml. of the patient's blood obtained with a dry-air sterilized syringe are placed in a clean dry test-tube and allowed to stand for twenty-four hours in an incubator at 37° C., along with a tube of blood from a normal person. In cases of nocturnal hemoglobinuria, gross hemolysis is evident, while in other conditions none is seen.

The *bone marrow* is quite the same as one finds in severe chronic hemolytic anemias of any type, being solidly filled with hematopoietic tissue composed chiefly of erythroblasts and normoblasts. One slight point of differentiation is the relatively small amount of iron-bearing pigment present in the tissues at large, while large quantities are deposited in the kidneys, especially along the convoluted tubules and the ascending loops of Henle.

Treatment is restricted to general hygienic measures. Splenectomy does no good, and blood transfusions are apt to be followed by hemolytic reactions Administration of alkalis may inhibit hemolysis briefly, only to be followed by accelerated red cell destruction. Even so, some patients have lived from fifteen to thirty years after the disease became manifest

BLOOD-TRANSFUSION REACTIONS

In view of the excellent monographs on blood transfusion that are available,† only

* Arch. Int Med., 64 1271 1939

† DeGowin and associates Blood Transfusion, W B Saunders Co, and Wiener, A S Blood Groups and Transfusion, Charles C Thomas

brief consideration will be given the subject, limited to mention of inter- and intragroup hemolytic reactions.

Intergroup Reactions. The severity of the reaction which follows transfusion of incompatible blood is dependent chiefly on (1) the titer of agglutinins in the recipient's plasma and (2) the amount of incompatible blood given. Most of the hemolysis that results is due to the action of the recipient's agglutinins on the donor's cells; it would be rare to find such a high agglutinin titer in the donor's plasma that significant damage to recipient's cells would ensue, taking the dilution factor into consideration. I know of a group B patient having been given 500 ml. of AB blood without even an elevation in temperature following the transfusion. At the other end of the scale, as little as 100 ml. of incompatible blood may cause death in a week or ten days from renal failure.

Symptoms indicative of incompatibility are usually manifest soon after the transfusion is started, notably restlessness, precordial oppression, tingling sensations, pain in the back and legs, and flushing. The pulse and respiratory rate are increased. The patient may go into shock, or may develop chills followed by high fever In some cases, symptoms are delayed, and when the patient is comatose or under anesthesia, or in deep shock (Fig 132), they may be overlooked entirely and the full half-liter given, adding to the seriousness of the situation.

Fully half of the patients receiving incompatible blood will die, a few rather promptly from the initial shock, but the majority after a week or two from damage to the kidneys (lower nephron nephrosis) The sequence of events in the latter group is as follows intravascular agglutination of erythrocytes, breakdown of agglutinated cells, hemoglobinemia, hemoglobinuria, oliguria, anuria, uremia, and usually death An occasional patient has been tided over the period of renal failure until kidney function was once more resumed

Once a transfusion reaction has occurred, it is imperative to determine immediately whether it was hemolytic * The blood group of donor and recipient must be rechecked and the crossmatch repeated The patient's blood serum should be examined for oxyhemoglobin within the first few hours after the reaction, and the serum bilirubin level followed over the next two or three days. The urine should be watched for evidence of hemoglobinuria during the first day and for haem casts thereafter; albuminuria may also be noted.

* Mollison Brit M J, *1* 529, 559, 1943

When fair evidence of hemolysis has been adduced, careful records of fluid balance must be kept and the blood repeatedly checked for evidence of nitrogen retention. Should kidney failure supervene, the blood phenol level is a fairly reliable prognostic index, if it remains below 4 mg. per 100 ml the outlook is rather good, even though the blood urea nitrogen may exceed 100 mg., but if it exceeds 6 mg. there is virtually no chance of recovery.

Treatment consists in promptly rendering the urine alkaline, although some doubt has been expressed as to just how much good this does. It is best accomplished by the intravenous administration of one-sixth molar solution of sodium lactate. Generous quantities of fluids should be given In the event of complete renal failure, peritoneal lavage or use of the artificial kidney may be considered.

Intragroup Reactions. These may be divided as apparent and real. Apparent intragroup reactions may actually be intergroup incompatibilities, resulting from complexities introduced by A subgroups. For example, a weak group B test serum (having a low titer of α) may not react with agglutinogen A_2, so that the blood will mistakenly be grouped as O

Real intragroup reactions are mostly centered around the Rh factor. An Rh negative recipient may become sensitized to Rh positive erythrocytes by repeated transfusions of Rh positive blood or, in the case of females, by carrying or having given birth to an Rh positive offspring When the reactions are due to previous blood transfusions, they are generally mild or moderately severe (Fig 133), and the symptoms develop relatively slowly, even in those few that terminate fatally. Anti-Rh sensitization incident to pregnancy usually results in a more serious reaction to transfusion of Rh positive blood.

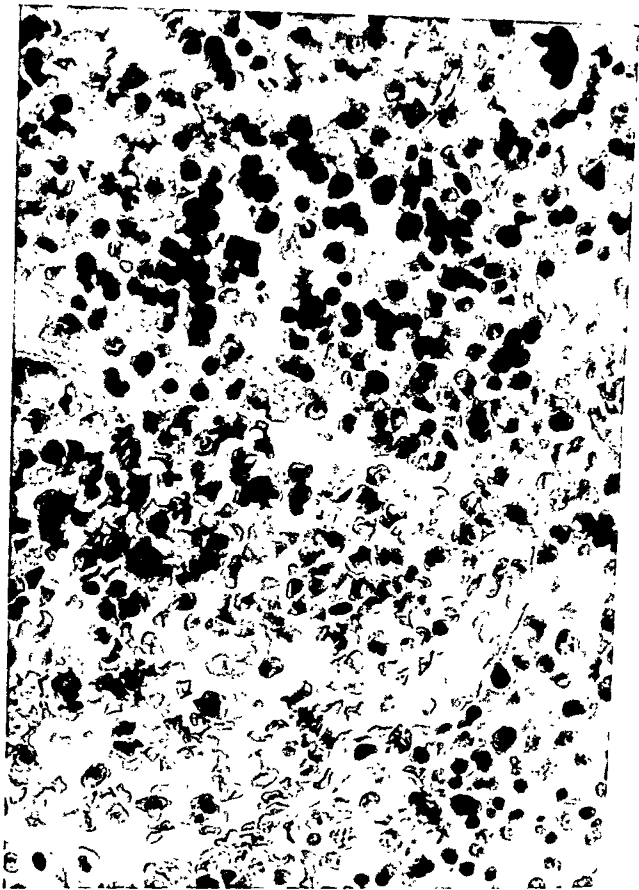


Fig 132 *Blood-Transfusion Reaction, Inter-Group Bone Marrow Section* A virtually exsanguinated and severely shocked patient of blood Group B was given a 500 ml transfusion of Group AB blood, the emergency apparently did not permit time for crossmatching, which should have revealed the error The patient gave no evidence of an untoward reaction during the transfusion, but died shortly after it was completed The illustration shows a large blood sinus of the marrow containing masses of agglutinated erythrocytes, the same phenomenon was noted in blood vessels of all tissues sectioned (× 1000)

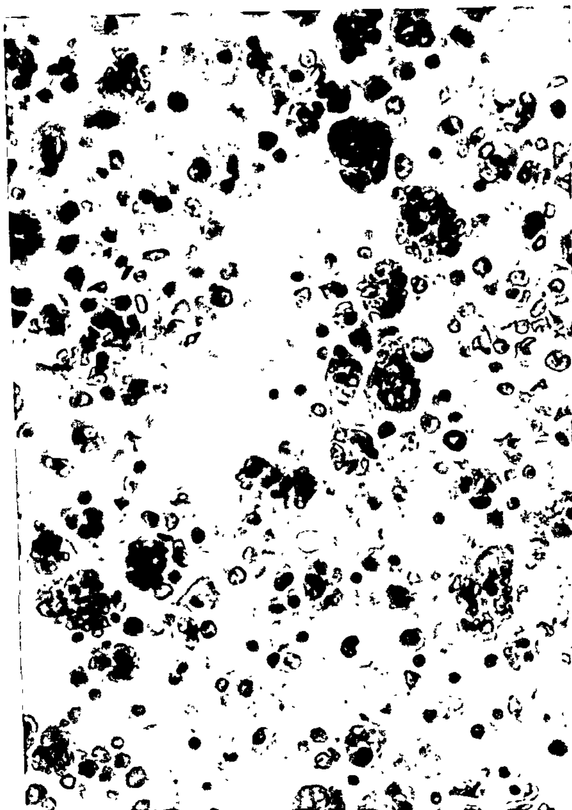


Fig 133 *Blood-Transfusion Reaction, Intragroup Bone Marrow Section* This patient had aplastic anemia and received a series of blood transfusions in quick succession Although crossmatching disclosed no incompatibility of donated blood, the patient experienced chills and fever after most of the transfusions Conditions were such that the Rh factor could not be determined in either donors or recipient At autopsy cells of the reticulo-endothelial system throughout the body were hyperplastic and heavily laden with whole or fragmented erythrocytes This is particularly conspicuous in the marrow, which is otherwise empty, apart from a scattering of lymphocytes and plasmacytes (× 1000)

Much of what has been said of intergroup reactions is applicable here also.

The "Universal Donor." Many serious hemolytic reactions have resulted from the misapprehension that group O blood can be indiscriminately administered to anyone, irrespective of his blood group or subgroup, as the term "universal donor" would imply. Some of the reactions have been due to anti-Rh sensitization, the others mostly to fairly high titers of agglutinins α and β in the group O blood. The only safe means of using group O blood for recipients of other groups is (1) to neutralize agglutinins α and β with group-specific substances A and B (Witebsky substance), and (2) to insure Rh compatibility.

HEMOLYTIC ANEMIA DUE TO INFECTIONS

See Chapter XIV with reference to infectious mononucleosis (p. 207), infections with hemolytic cocci (p. 215), clostridia (p. 218), plasmodia (p. 228), and bartonellae (p. 240).

HEMOLYTIC ANEMIA DUE TO CHEMICAL AGENTS

See Chapter XII, page 184

HEMOLYTIC ANEMIA DUE TO PHYSICAL AGENTS

See Chapter XII, pages 169 and 171.

HEMOLYTIC ANEMIA DUE TO ALLERGY

Hypersensitivity to certain vegetable substances is responsible for the development of acute hemolytic episodes following exposure to the allergenic material. The two most commonly recognized are the fava bean or its pollen, and the pollen of certain spring flowers in the neighborhood of Baghdad.

Favism. Thus far, most cases of favism have been limited to Sardinia, Sicily, and Italy, but the extensive importation and cultivation of fava beans in this country, along with a considerable population of Mediterranean peoples, make it likely that the condition may appear in more than the few isolated instances already reported.

Attacks of hemolytic anemia due to inhalation of the pollen occur in the late spring, whereas those following ingestion of the bean may be seen at any time of year. From five to twenty-four hours after exposure to the allergen the patient becomes dizzy, feverish, and may even collapse. Pallor, jaundice, and hemoglobinuria follow rapidly. The erythrocyte count falls to 1 to 2,000,000 per cu. mm., and marked leukocytosis is noted during the first week. Clinical recovery is fairly rapid, but jaundice may persist for several weeks and anemia still longer. As in other forms of hemolytic anemia, the bone marrow displays erythropoietic hyperplasia, reflected in the peripheral blood by reticulocytosis and occasional nucleated red cells

Baghdad Fever. The manifestations of this allergic state are similar to those of favism, the allergen being the pollen of flowering plants which is dispersed during the spring months.

HEMOLYTIC ANEMIAS ASSOCIATED WITH MALIGNANT TUMORS

See Chapter VII, page 89, and Chapter X, page 151.

X

ILL-DEFINED ANEMIAS

There are certain conditions in which we do not understand the precise mechanism by

tory anemias" because, not only is the cause unknown, but they resist our present means of treatment. A few examples of each class will be considered

CACHEXIA OF MALIGNANT DISEASE

Cachexia is an ancient term (from *kakos*, bad + *hexis*, condition), appropriately designating the late stages of malignant disease in which anemia is a feature. The tumor may be responsible for loss of blood, displacement of bone marrow, or interference with nutrition. Recent experiments have indicated that extracts of cancerous tissue exert a hemolytic effect not produced by extracts of normal organs. This may explain the occasional occurrence of acute hemolytic anemia in connection with malignant tumors Carcinoma of the stomach, if sufficiently extensive, can also restrict production of the intrinsic factor. Any or all of these conditions may obtain in a patient.

The anemia is generally microcytic and hypochromic when chronic bleeding is the principal cause, although some cases of cecal carcinoma with large oozing surfaces have shown pronounced macrocytosis Macrocytic anemia is also seen in patients where nutritional deficiency, hepatic insufficiency, or lack of intrinsic factor occur. I have seen carcinoma of the stomach or cecum simulate pernicious anemia so closely that bone marrow study and therapeutic trial of liver extract were employed in the differential diagnosis, roentgenologic examination of the gastro-intestinal tract having been inconclusive. Extensive metastasis to bones is followed by myelophthisic anemia, usually normocytic and occasionally leukoerythroblastic in type (p. 101), although this seldom happens.

Biopsy of the bone marrow may fortuitously strike a site of tumor, making the diagnosis easy. (See illustrations in Chapter IX.) Otherwise, one will encounter either hyperplastic hematopoietic tissue, especially of the erythrocytic series and frequently with eosinophilia, or so-called "gelatinous degeneration" in exhaustion states Under any circumstances, it is a "bad condition."

ANEMIAS RESULTING FROM CHRONIC RENAL DISEASE

Retention of the nitrogenous products normally excreted by the kidneys (chronic glomerulonephritis, chronic pyelonephritis, congenital polycystic kidneys, some cases of malignant nephrosclerosis), if sufficiently prolonged, is invariably followed by anemia, the severity of which parallels the degree and duration of the uremia. Anemia is generally normocytic, and may be normochromic or hypochromic depending on the nutritional state of the patient There is little evidence of blood regeneration, the reticulocyte count being low and poikilocytosis inconspicuous; an unusually wide variation in red cell diameter is frequently noted, however (Fig. 134). Neutropenia is often associated with the erythrocyte depression The thrombocyte count remains within

Fig. 134. *Chronic Glomerulonephritis. Blood* Chronic nitrogen retention had progressed in this patient over many months, and was paralleled [illegible]

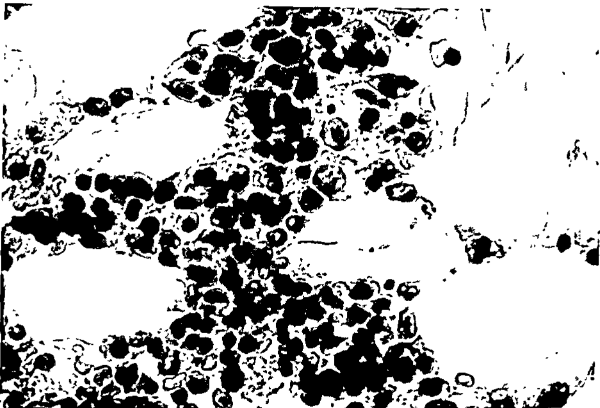


Fig 135 *Chronic Glomerulonephritis Bone Marrow Section* Sternal marrow removed at autopsy from the patient whose blood is shown in Fig 134 There is a relative erythropoietic hyperplasia, but the response is far less than one would expect in proportion to the degree of anemia present, as though some inhibiting influence had been exerted (× 1000)

normal limits, and the hemorrhagic phenomena commonly observed in uremia are probably due to damaged capillary endothelium.

At best, the bone marrow is normally cellular, more frequently being hypoplastic. The erythrogranulocytic ratio is usually inverted, so that red cell progenitors predominate, with most in the later stages of maturation (Fig. 135). Poorly nourished patients may also display gelatinous degeneration of the marrow (Fig 136).

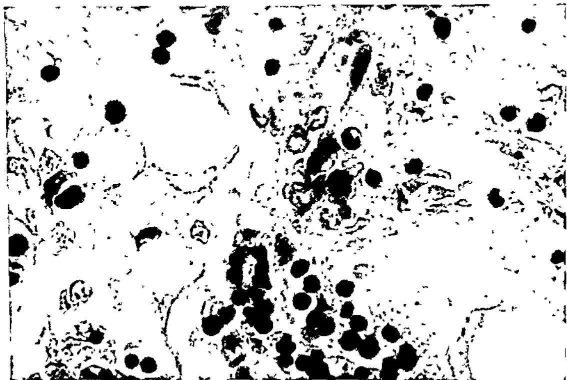


Fig 136 *Chronic Glomerulonephritis Bone Marrow Section* The degree of anemia presented by this patient was still more marked than in the preceding case, and was distinctly hypochromic in type General nutrition was also poorer, as reflected in the marrow which shows hypoplasia and serous atrophy (starvation change) Most of the residual cells are later stages of the erythrocytic series This case illustrates the added influence of malnutrition on the severity of anemia in chronic nitrogen retention (× 1000)

BANTI'S SYNDROME

So-called "Banti's syndrome" (splenic anemia) is seldom seen beyond the age of thirty-five, and frequently becomes manifest during childhood. There may be a history of vague gastro-intestinal symptoms, or sudden gastric hemorrhage may occur without warning. The spleen is moderately to markedly enlarged, sometimes in association with hepatomegaly. Examination of the *blood* discloses a mild normocytic anemia, leukopenia with a normal differential count, and frequently a decrease in the number of thrombocytes. Anemia may become severe and hypochromic if gastric bleeding is prolonged or repeated at short intervals, and may be macrocytic during the regenerative phase that follows a sudden massive hemorrhage.

The *bone marrow* generally shows little significant change when there is no bleeding, although granulocytic hyperplasia has been described Hemorrhage is followed by normoblastic hyperplasia (Fig 137) Patients with advanced liver disease may present considerably greater immaturity in the erythrocytic series, in which case the anemia is apt to be macrocytic. Megakaryocytes are often increased in number, but are qualitatively normal.

It is generally accepted that Banti's syndrome is the consequence of chronic hyper-

tension in the splenic vein, irrespective of the means by which this hypertension is brought about. In a good many cases, the cause of the splenic or portosplenic hypertension can be demonstrated, notably cirrhosis of the liver or changes in the splenic or portal veins (endophlebitis, thrombosis). No apparent cause was found in other instances. I have seen a case in which painstaking dissection of the vessels at the splenic hilum revealed a large arteriovenous anastomosis; this had obviously increased intrasplenic pressure. This type of anomaly would serve to explain at least some of the "idiopathic" cases, and would also account for the syndrome frequently developing in early childhood.

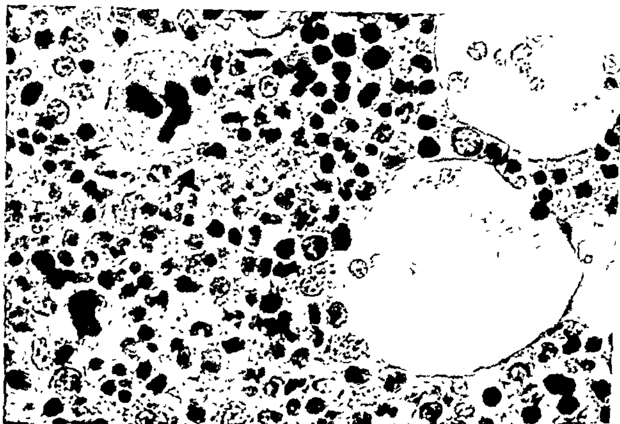


Fig. 137. *Banti's Syndrome. Bone Marrow Section.* The patient was an eleven-year-old girl who had had several episodes of hematemesis and whose splenic edge reached the level of the umbilicus. Examination of the peripheral blood disclosed a mild hypochromic anemia, leukopenia with a normal differential count, and moderate thrombocytopenia. Section of sternal bone marrow shows cells with round dark nuclei (erythroblasts and normoblasts) to predominate, indicating a relative and absolute hyperplasia of red cell progenitors in response to the anemia; megakaryocytes are also prominent (× 1000).

ANEMIAS OF PREGNANCY

A moderate *normocytic anemia* occurs in a sufficiently large percentage of pregnant women that some authors regard it as physiological. Hydremia appears to be largely responsible for the decreased red cell count. *Hypochromic anemia* due to dietary deficiency (especially iron) is also common and differs in no way from the iron deficiency anemia already described (p. 43).

Pernicious Anemia of Pregnancy. See page 59.

REFRACTORY ANEMIAS

The term "refractory anemia" has been used by some authors to include all types of aregenerative anemia which do not respond to treatment other than transfusion of whole blood. This would encompass idiopathic aplastic anemia (p. 171), secondary aplastic anemia (p. 74), myelophthisic anemias (p. 81), certain unclassified chronic hemolytic anemias (p. 139), achrestic anemia (p. 68), and the paradoxical aplastic anemia with hyperplastic

marrow. It is perhaps better to limit this group to the last two. Achrestic anemia has already been considered, and there is little to differentiate it from aplastic anemia with hyperplastic marrow, a designation which has served only to confuse the issue since it was proposed by Rhoads and Miller.

The anemia is generally macrocytic, occasionally normocytic, with a relatively low reticulocyte count which is not significantly altered by injections of potent liver extract Neutropenia and thrombocytopenia are usually associated Histamine-fast achlorhydria may or may not be present. The bone marrow is hyperplastic, and megaloblastic in most cases showing macrocytosis

Davidson* reported a series of cases under the heading "refractory megaloblastic anemia" in which satisfactory remission followed administration of either folic acid or so-called "proteolysed liver."

* Blood, 3 107, 1948.

XI

HEMORRHAGIC STATES

Menses and childbirth are the only conditions in which bleeding (within limits) can be regarded as normal. Even these can exceed normalcy to produce anemia or even death. Other causes of hemorrhage are listed in Table 15.

TABLE 15

CAUSES OF HEMORRHAGE

I *Trauma*
- (A) Direct injury to blood vessels
- (B) Erosion of blood vessels due to various pathological processes

II *Defects in Coagulability of the Blood*
- (A) Deficiency in number of thrombocytes, due to
  - (1) Decreased production
    - (a) Idiopathic thrombocytopenic purpura
    - (b) Other forms of hypersplenism (Banti's and Felty's syndromes, etc)
    - (c) Pernicious anemia (some cases)
    - (d) Aplastic anemia
    - (e) Leukemias
    - (f) Other myelophthisic states
    - (g) Chemical agents (direct, allergy) (see Table 17)
    - (h) Physical agents (radiant energy, burns, heat stroke, fever therapy)
    - (i) Infections
  - (2) Increased destruction
    - (a) Idiopathic thrombocytopenic purpura (?)
    - (b) Other forms of hypersplenism (?)
    - (c) Chemical agents (allergy, especially sedormid, organic arsenicals)
    - (d) Infections
  - (3) Excessive loss
    - (a) Thrombotic thrombocytopenic purpura
- (B) Deficiency of a plasma factor
  - (1) Prothrombin
    - (a) Hemorrhagic disease of newborn
    - (b) Idiopathic hypoprothrombinemia
    - (c) Inhibited bacterial flora of intestines (sulfasuxidine, etc)
    - (d) Impaired absorption from intestines
    - (e) Dietary deficiency
    - (f) Severe liver damage
    - (g) Dicumarol (therapeutic, sweet-clover disease of cattle)
    - (h) Salicylates
  - (2) Fibrinogen:
    - (a) Congenital
    - (b) Severe liver damage
  - (3) "Fraction I" of Cohn:
    - (a) Hemophilia
- (C) Excess of anticoagulants:
  - (1) Heparin·
    - (a) Therapeutic
    - (b) Anaphylactoid shock
    - (c) Peptone shock
  - (2) Hirudin (leech extract)
  - (3) Unidentified

III *Vascular Abnormalities*
- (A) Congenital
  - (1) Pseudohemophilia (hereditary hemorrhagic thrombasthenia)
  - (2) Multiple hereditary telangiectasia
- (B) Increased capillary permeability, due to.
  - (1) Idiopathic nonthrombocytopenic purpura
  - (2) Allergenic nonthrombocytopenic purpura
    - (a) Bacterial (infection or vaccine)
    - (b) Chemical (see Table 17)
    - (c) Vegetable (foods, poison oak, etc)
  - (3) Shock
  - (4) Uremia
  - (5) Venoms
  - (6) Vitamin deficiencies:
    - (a) Vitamin C
    - (b) Vitamin P (rutin)

IV *Intravascular Phenomena*
- (A) Embolism (especially subacute bacterial endocarditis)
- (B) Capillary thrombosis
  - (1) Erythremia (polycythemia rubra vera)
  - (2) Thrombotic thrombocytopenic purpura

V *Endocrine Disorders*
- (A) Hormonal imbalances resulting in abnormal uterine bleeding·
  - (1) Pathologic endometrial hyperplasia
  - (2) Endometrial atrophy
- (B) Hormonal dysfunction resulting in generalized purpura
  - (1) David's disease

VI. *Diseases of the Skin*
- (A) Danlos's syndrome
- (B) Purpura annularis telangiectodes (Majocchi's disease)
- (C) Pigmented purpuric lichenoid dermatitis
- (D) Progressive pigmentary dermatosis (Schamberg's disease)

biopsies, both aspiration and trephine types, on "bleeders" without having encountered serious trouble. It is important to prepare tissue sections of aspirated marrow fragments (p. 307) in addition to the usual smears, so that the relative proportions of megakaryocytes to other cellular elements can be more accurately estimated.

most other conditions, the red and white cell progenitors react in conformity to the degree of blood loss and inflammation, so that interest usually centers about megakaryocytes, which may display qualitative or quantitative changes, or both.

Idiopathic Thrombocytopenic Purpura. The low thrombocyte level of this disease is due to

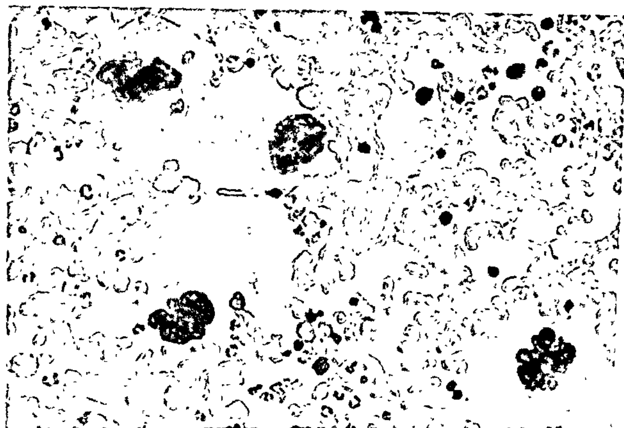


Fig. 138. *Idiopathic Thrombocytopenic Purpura Bone Marrow Smear.* The patient, a young woman who had bruised easily all her life, suddenly developed marked menorrhagia, epistaxis, bleeding from her gums, and spontaneous ecchymoses in her skin Thrombocytes were few, clot retractility absent, bleeding time increased, and tourniquet test positive. Sternal aspiration disclosed striking megakaryocytic hyperplasia (four in the field shown here) with sparse cytoplasmic granulation and poor or absent thrombocyte margins. Splenectomy effected a rapid clinical cure (× 500)

Aplastic Anemia and Acute Leukemias. The most readily recognizable marrow pictures are observed in aplastic anemia and the acute leukemias, where the myeloid cavity is respectively empty or overfilled with immature leukocytes, and where the outlook is virtually hopeless. The megaloblastic marrow of pernicious anemia, poor in megakaryocytes, is easily identified and the deficiency in hematopoietic principle corrected without difficulty. The marrow in Gaucher's disease will contain the typical cells, while in certain infections (notably kala-azar), organisms may be recovered In

greatly diminished thrombocyte production by megakaryocytes (which can only be evaluated in smears), even though the number of these cells in the marrow is increased over normal, sometimes strikingly so (Figs. 138, 139, 140). Dameshek and Miller* put the normal number at a maximum of 300 per million nucleated red blood cells, with an average of 68.8 per cent showing thrombocyte production, while their cases of idiopathic thrombocytopenic purpura ranged from 366 to 743, and only from 8 to 19 per cent were forming

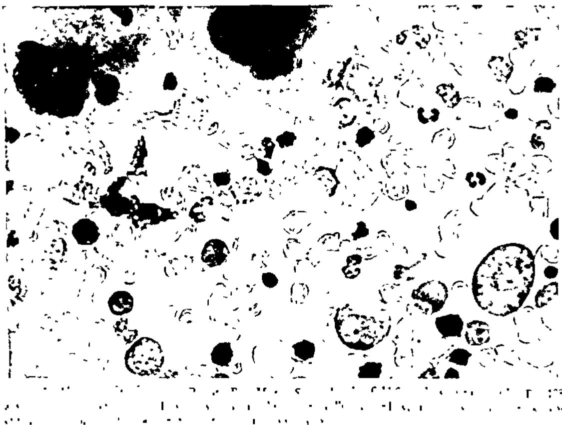
* Blood, 1 27, 1946.

thrombocytes. There also is a poverty or absence of granules in the cytoplasm of a good many megakaryocytes This is the type of case in which splenectomy is generally (not invariably) followed by a striking increase in thrombocyte production, rapid restoration of thrombocytes to normal or above, and prompt relief from hemorrhage. Sometimes the bleeding stops, but the rise in thrombocytes is slow; occasionally splenectomy fails in both respects.

Allergenic, Toxic, and Infectious Thrombocytopenic Purpura. In the allergenic, toxic or infectious types of thrombocytopenic purpura, the megakaryocytes are selectively affected They may be present in normal, increased, or diminished numbers, but usually show evidences of degeneration such as pyknosis or loss of nuclei (Fig 141).

Eosinophilia of the marrow is generally found in the allergenic group Splenectomy has nothing to offer under these circumstances, and spontaneous recovery will often follow identification and removal of the offending substance.

Thrombotic Thrombocytopenic Purpura. This disease cannot be diagnosed from smears of aspirated bone marrow. Sections of aspirated fragments, however, will disclose capillaries and even large blood sinuses occluded by granular acidophilic thrombi containing little or no fibrin (Figs. 142, 143). The marrow in general is hyperplastic, and megakaryocytes appear in usual or increased numbers and are



structurally normal No form of therapy has proved effective in this condition.

Nonthrombocytopenic Purpura. The bone marrow in nonthrombocytopenic purpuras does not display any very remarkable change, and the biopsy may prove valuable from this negative standpoint. Nonspecific hyperplasia of erythrocyte progenitors may be noted, and megakaryocytes occasionally are increased in numbers (Fig 144); *they show normal* thrombocyte formation. Marrow eosinophilia is frequently present (Fig 144).

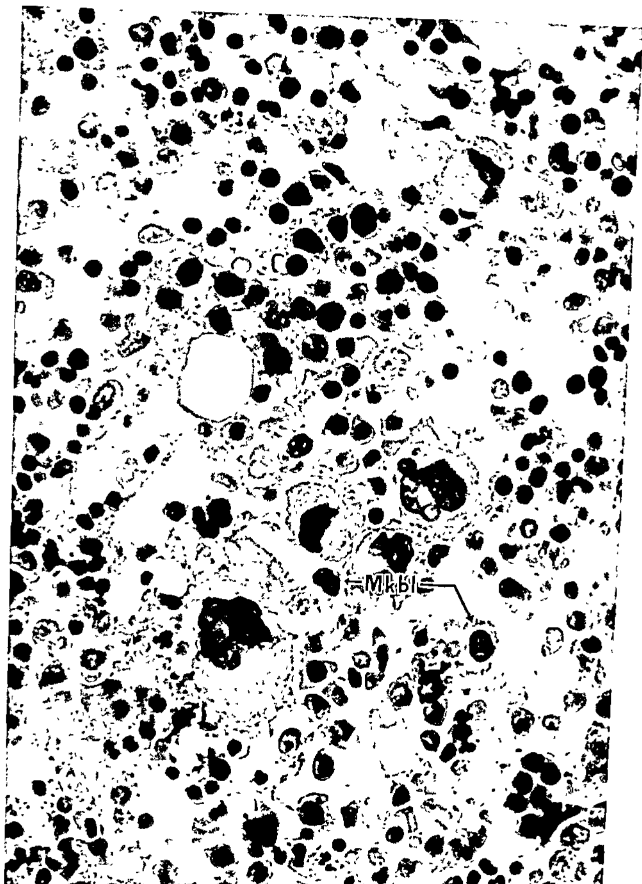


Fig 140 *Idiopathic Thrombocytopenic Purpura Bone Marrow Section* The patient was a white woman of thirty-two years who presented classical clinical and hematologic features of the disease She had lost large quantities of blood with her menses for nearly a year and had a pronounced hypochromic microcytic anemia Section through an aspirated fragment of sternal bone marrow shows five megakaryocytes and at least two megakaryoblasts (Mkbl) in the field Most of the other cells belong to the erythrocytic series (× 1000)

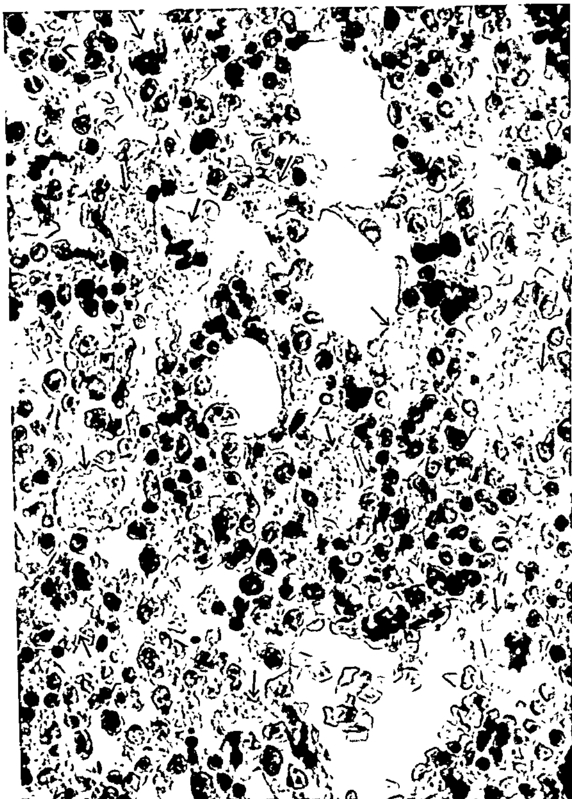


Fig 141 *Allergenic Thrombocytopenic Purpura Bone Marrow Section.* A Negro soldier developed rheumatic fever while [illegible]

Fig 142. *Thrombotic Thrombocytopenic Purpura Bone Marrow Section.* A young woman recently pregnant developed severe hemorrhagic purpura, with anemia, neutrophilic leukocytosis, and thrombocytopenia She died after a few weeks, and autopsy disclosed widespread thrombosis of arterioles, capillaries, and venules The thrombi, as shown in marrow capillaries here, were granular, acidophilic, and poor in fibrin. They are presumably composed largely of thrombocytes. The marrow is diffusely hyperplastic and contains the usual complement of megakaryocytes (not shown in this field) (× 1000).



Fig 143 *Thrombotic Thrombocytopenic Purpura Bone Marrow Section* Same case as Fig 142. This section shows similar thrombosis of a large blood sinus of the marrow (× 250)

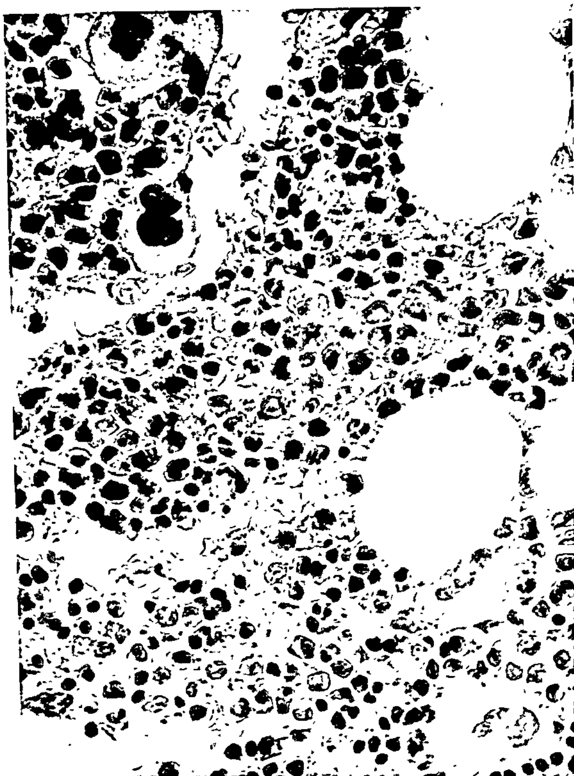


Fig. 144. *Allergenic Nonthrombocytopenic Purpura. Bone Marrow Section.* A park laborer who had been exposed to poison oak developed purpuric blotches at contact points one day later. Generalized skin purpura spread rapidly, and he began to bleed from mucous membranes within a few days. Examination of the blood showed moderate leukocytosis with 10 to 20 per cent of eosinophils, but no anemia or thrombocytopenia. He died on the tenth day. The marrow was moderately hyperplastic with a slight increase in number of normal megakaryocytes and a minor eosinophilia. The field illustrated contains four megakaryocytes, the two in the upper left are interesting in that they occupy a shoreline position along a blood sinus and present pseudopodial intrusions into the lumen (J. H. Wright's original explanation of thrombocyte delivery). Coarse granules mark the eosinophils, most prominent near the center (× 1000).

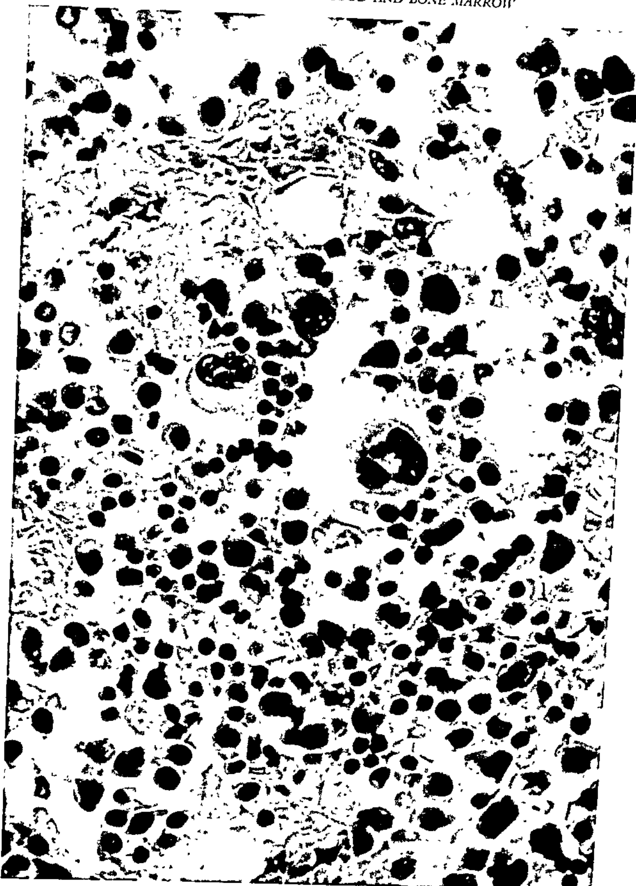


Fig. 145 *Hemophilia Bone Marrow Section* An eleven-year-old Italian boy from a hemophilic family died of massive

Hemophilia Krumbhaar and I* described our findings in three fatal cases of hemophilia The bone marrow was normally reactive and contained a significantly increased number of normal megakaryocytes and their progenitors (Fig. 145). I have not studied the marrow from cases of pseudohemophilia, nor have I found descriptions in the literature; it is safe to assume, however, that no specific alteration exists.

pressure, and markedly decreased cardiac output, later by accelerated pulse, faintness, sweating, and nausea on slight effort Loss of more than 1500 ml. is likely to cause death. If the hemorrhage is prolonged over twenty-four hours, however, a patient can lose about *half* his blood and survive. The sequence of changes which occur in the peripheral blood following acute hemorrhage may be listed in the following manner.

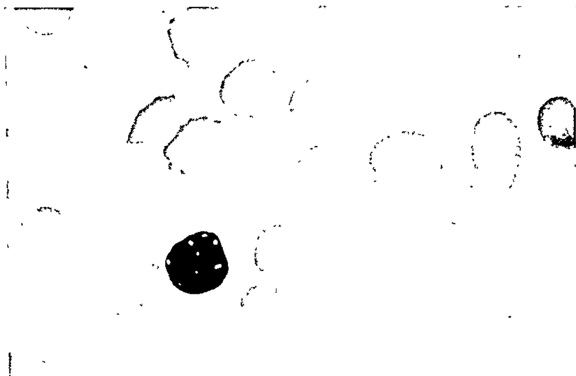


Fig. 146 *Chronic Hemorrhagic Anemia. Blood* The clinical notes on this case are found in the legend of Fig 147. Erythrocytes are exceedingly small, in contrast to the nucleated red cell and the reticulocytes in the right lower quadrant of the picture (× 2280)

POSTHEMORRHAGIC ANEMIA

The comments which follow are generalities applicable to hemorrhage per se from whatever cause. They are more apt to fit Group I (trauma to or erosion of blood vessels), however, for many patients with so-called "hemorrhagic diatheses" do not lose enough blood to produce anemia.

Acute blood loss up to 500 ml is withstood well by a healthy adult, particularly if he remains recumbent for a time Rapid loss of a liter is followed by a slow pulse, low blood

* Am J M Sc, *189* 620, 1935

Thrombocythemia	Within 1 hour
Decreased coagulation time	Within 1 hour
Neutrocytosis, sometimes leukemoid	Peak in 3–5 hours
Anemia	1–2 days
Reticulocytosis	1–2 days

The lag in appearance of anemia is due to vasoconstriction and redistribution of remaining blood, as well as the time required to restore blood volume from tissue fluid The anemia which follows is usually normocytic and normochromic, sometimes briefly macrocytic when reticulocytosis is pronounced. Reticulo-

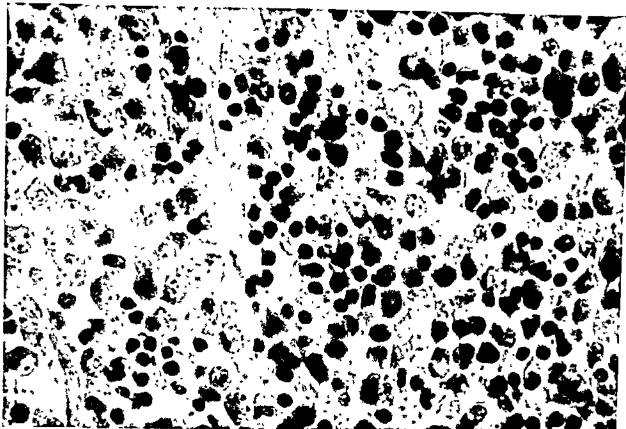


Fig 147 *Chronic Hemorrhagic Anemia. Bone Marrow Section* An elderly woman who complained of weakness was found to have a red cell count of 4,000,000 per cu. mm. and 4 gm of hemoglobin per 100 ml Examination revealed internal hemorrhoids which bled slightly on manipulation. Prompt recovery followed administration of iron and surgical care of the hemorrhoids The marrow is solidly cellular, with the majority being late forms of nucleated red cells (× 1000)

cytes should reach normal during the second week; if they remain elevated, one should suspect continued or recurrent bleeding. Erythrocytes attain normal level in about a month, while hemoglobin is restored a little more slowly. Leukocytosis seldom persists longer than three to five days, unless there has been hemorrhage into one of the serous cavities of the body or an associated infection. Bone marrow hyperplasia necessary to compensate for an acute hemorrhage is minimal.

Chronic bleeding, however slight, will gradually deplete the iron stores of the body and will ultimately result in hypochromic anemia which is generally microcytic (Fig. 146) Symptoms are often remarkably few, perhaps only tiredness and pallor, as the progress of the anemia may be so slow that the patients learn to live with their hypotoxic state Clinical and hematologic data are similar to those of other iron deficiency anemias and have already been detailed (pp. 43–45). The bone marrow is strikingly hyperplastic, with red cell progenitors predominating, mostly in the later stages of maturation (Fig 147). Hyperplasia of granulocytes and megakaryocytes may be present.

XII

EFFFCTS OF PHYSICAL AND CHEMICAL AGENTS

HEAT

Burns

Human blood heated to from 47° to 65° C. (116 6° to 149° F.) was shown by Ham and co-workers* to undergo striking morphologic changes in the erythrocytes, notably division with development of spheroid cells which were osmotically and mechanically fragile to a marked degree. This observation offers an explanation for the hemolytic anemia frequently observed in humans after severe thermal burns, sometimes to the point that hemoglobinuric (lower nephron) nephrosis develops Since plasma remains relatively isotonic, probably the increase in mechanical fragility is responsible for the abnormal red cell destruction. Direct observation of the peripheral blood (Fig 148), especially wet preparations, generally discloses a significant degree of spherocytosis Serum bilirubin is usually elevated and,

* Blood, *3* 373, 1948.

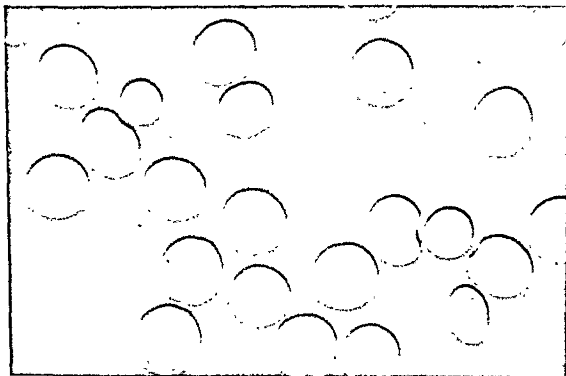


Fig 148. *Burn. Blood* Moderate degree of spherocytosis is evident, especially in the cells of smallest diameter This patient had a mild anemia, and an increase in serum bilirubin and urobilinogen (× 2280)

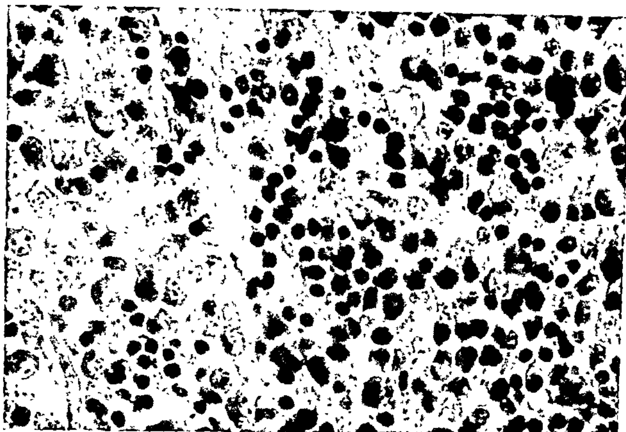


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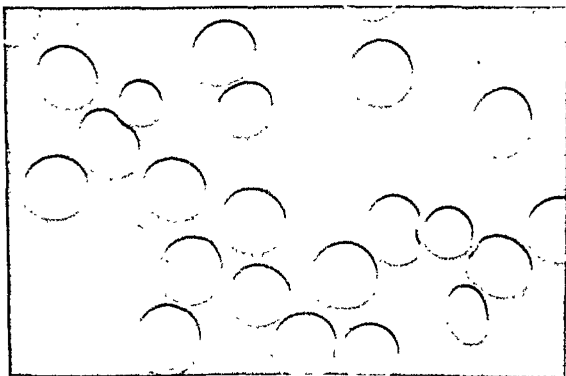


Fig 148. *Burn Blood* Moderate degree of spherocytosis is evident, especially in the cells of smallest diameter This patient had a mild anemia, and an increase in serum bilirubin and urobilinogen (× 2280)

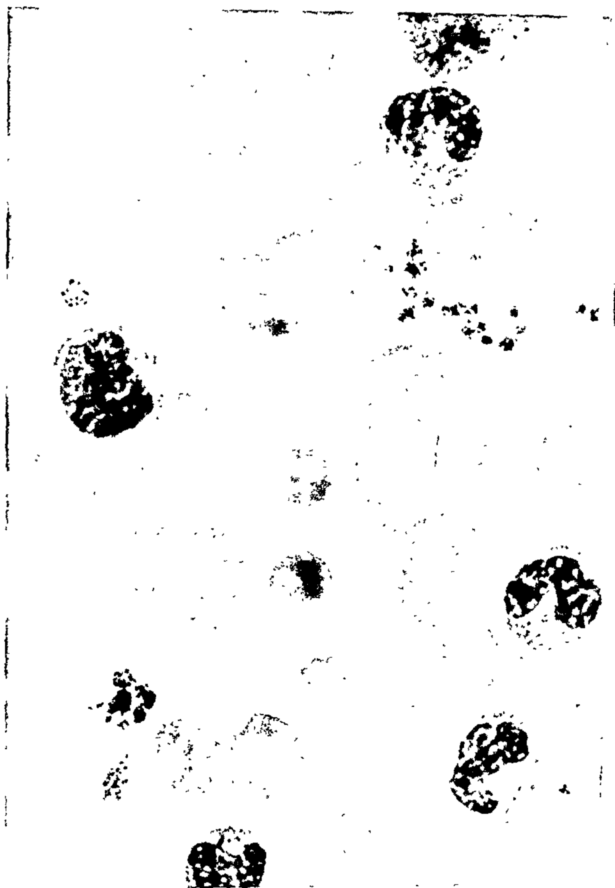


Fig 149 *Burn Blood* Leukemoid reaction, with 42,000 leukocytes, virtually all neutrophils, and many young forms in the peripheral blood (× 2280)

in cases sufficiently severe that hemoglobinuria has damaged the kidneys, oliguria and nitrogen retention occur.

Leukemoid reactions occasionally follow extensive burns (Figs. 149, 150); the few cases that I have observed were marked by third degree burns which were invariably fatal These high leukocyte levels, in one instance reaching 60,000 per cu. mm., were apparently stimulated by products of tissue breakdown rather than infection, as the counts rose before there was time for infection to supervene. Many immature neutrophils appeared in the peripheral blood, and the bone marrow was left edematous and relatively devoid of cells of the granulocytic series (Fig. 150). The thrombocyte count was also increased in some instances; other patients had a period of thrombocytopenia associated with purpura.

Heat Stroke

In our series of 125 fatal cases of heat stroke,* we gained the impression that the extent of hemorrhage was greater than that ordinarily seen in shock due to other causes. Hematologic data were sparse, but in the ten cases in which thrombocyte counts had been performed all were less than normal, the lowest being 22,300 per cu. mm Among thirty-five cases, the red cell count was below 4,600,000 in eighteen, and only two showed evidence of hemoconcentration with values over 6,000,000 per cu. mm Most patients dying within twenty-four hours had a leukocytosis ranging from 10,000 to 29,000, whereas few uncomplicated cases beyond this period had a significant rise Leukocytosis was due to an increase in neutrophils

Bone marrow was available for study in fifteen cases, only two of which were regarded as normal. The most consistent finding was degeneration of megakaryocytes, which we regarded as a direct effect of heat (Fig. 151) An actual increase in number of these cells was noted in some instances, as well as the occasional appearance of young forms Depletion of cells of the granulocytic series and/or nucleated red cells occurred in about one-third of these patients.

* Mil Surg, 99 397, 1946

COLD

Direct Effects

Injury produced by freezing, and more especially by prolonged chilling, results in breakdown of fat cells in the bone marrow of the affected part. Liberated lipids primarily form pools in the marrow spaces, but there is a rapid influx of macrophages which engulf the fatty material, and the myeloid cavity is soon filled with diffuse sheets of large foam cells (Fig 152). Fibrous tissue is gradually laid down to replace the lipophages ultimately, and layers of new bone frequently marginate the original trabeculae of the cancellous bone (Fig 153). The myelofibrosis is not reflected in the peripheral blood, because it is the bones of the extremities which are invariably involved, and the marrow of these bones is not normally concerned with blood formation.

Activation of Hemolysins

Sudden intravascular destruction of red blood cells sometimes occurs during the warming period from a few minutes to some hours after exposure to cold. Such a phenomenon is due to the action of an autohemolysin which unites with the red cells only at low temperatures. This type of *paroxysmal hemoglobinuria* occurs nearly always in syphilitics (usually congenital), and the hemolysin is thought to be an antibody similar to reagin of the Wassermann reaction The hemolytic episode generally begins with generalized aches and low back pain, followed by a short period of chills and fever. The next few specimens of urine are usually mahogany brown to black. The patient is left weak, pallid, and often icteric. Attacks vary considerably in severity, recovery from each is mostly rapid, and in the intervening periods the patients may be quite well.

Examination of the blood discloses anemia and pigmentation of the plasma directly proportional to the severity of the attack; the pigments are chiefly hemoglobin, methemalbumin, and bilirubin Reticulocytosis follows promptly, and red cell levels are soon restored to normal. The leukocyte count is apt to be low during the period of exposure to cold, but neutrophilic leukocytosis of varying degree

Fig 150 *Burn. Bone Marrow Section* From the case shown in Fig 149, the marrow is depleted of cells of the granulocytic series, virtually all nucleated elements belonging to the erythrocytic series The interstices are the seat of edema and hemorrhage (× 1000)

Fig 151 *Heat Stroke Bone Marrow Section* There is a relative and actual increase in the number of megakaryocytes, nearly all of which are degenerated In the field illustrated, these cells are seen as large anuclear masses of cytoplasm, in other areas, some contain pyknotic or fragmented nuclei There is also a paucity of other normal elements of the marrow, especially cells of the granulocytic series, associated with interstitial hemorrhage (× 1000)

Fig. 152 *Cold. Bone Marrow Section.* Sheets of macrophages laden with lipids (foam cells) fill marrow spaces in which normal fat cells of the marrow have been disrupted by freezing or prolonged chilling (× 800).

Fig 153 *Cold Bone Marrow Section* Stage of organization, where marrow spaces are replaced by fibrous tissue, and trabeculae show marginal laminae of new bone (× 200)

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follows the hemolytic phase, frequently with the appearance of immature forms.

In common with any hemolytic anemia, *the bone marrow* displays hyperplasia of erythrocyte progenitors, most of which are late erythroblasts and normoblasts. The *hyperplasia* is rarely marked, however, because of the evanescent character of the anemia.

When hemoglobinuria follows exposure to cold, the *diagnosis* is clear. In milder cases, where hemoglobinemia does not exceed the renal threshold, it is necessary to employ the Donath-Landsteiner or modified Mackenzie tests; the principle behind each of these tests is the same; i e., demonstration of hemolysis after chilling and then warming a sample of the patient's blood. There is usually serologic or clinical evidence of syphilis, as well.

RADIANT ENERGY

Ionizing radiation from any source (alpha, beta, gamma, roentgen ray, or neutron) exerts a similar effect on living tissues in general when the amount absorbed (energy and time relationship) is the same in each instance. Hematopoietic elements of the bone marrow, more especially cells of the granulocytic series, are exceeded in radiosensitivity only by lymphatic tissue. Dosage, duration of exposure, and repetition of exposure determine to some extent the type and degree of change which takes place in the marrow. There is also an individual variation in the type of reaction. For example, Warren* mentions the cases of two chemists who worked for some years in the same laboratory and were exposed to comparable amounts of radioactive substances; they died within five days of each other, one of aplastic anemia and the other of granulocytic leukemia.

The effects of *single large doses* of ionizing radiation are well illustrated among the atomic bomb casualties Those who died within the first four days showed virtually a total loss of all hematopoietic cells. In patients who survived for several weeks, a marked proliferation of atypical reticulum cells was noted, along with a plasmacyte reaction, but with little tendency to differentiate toward normal blood cell progenitors. Still others who were probably not so heavily exposed displayed regeneration and even hyperplasia of their marrow, stemming in part from surviving hematopoietic elements and in part from proliferating reticulo-endothelium. The anemia was of the aplastic type, with more or less striking decrease in the erythrocyte, leukocyte, and thrombocyte counts; in less heavily irradiated persons, the leukocyte levels were generally restored to normal by the eighth or ninth week, and erythrocytes and thrombocytes were restored from the twelfth to sixteenth week.

Intermittent small doses of ionizing radiation may also cause aplastic anemia if exposure is frequent (Fig. 154) and over a considerable period of time. This is due to the cumulative action of radiation Thus, in successive exposures, the radiation required to produce tissue damage becomes less, and the time necessary for recovery becomes longer. Quite the opposite situation may also occur. Whether very small doses of radiant energy are sometimes stimulating to hematopoietic tissue, or whether regeneration following repeated small doses proceeds to actual neoplasia of the leukopoietic elements, the fact remains that the incidence of leukemia in radiologists is from eight to ten times as great as in the population at large. Irradiation of experimental animals renders them more susceptible to both spontaneous and transmitted leukemia

Continuous small doses of radiant energy are still more dangerous, exemplified in the case of ingestion of radium where the bombardment is ceaseless. Martland's study† of radium-dial painters who pointed brushes in their mouths showed that radioactive substances were deposited in the bones It was estimated that a dial painter who had 10 micrograms of radioactive substances in his entire skeleton would still be giving off 185,000 particles of alpha radiation per second in the year 3491 A D. A variety of changes resulted (Fig. 155). A severe macrocytic anemia simulating pernicious type, associated with megaloblastosis of the bone marrow, was regarded as the first stage of radiation osteitis. In patchy areas over the skeleton, a cellular fibroblastic replacement of the mar-

* Warren and Dunlap' Arch Path, *34* 562, 1942

† Am J Cancer, *15* 2435, 1931

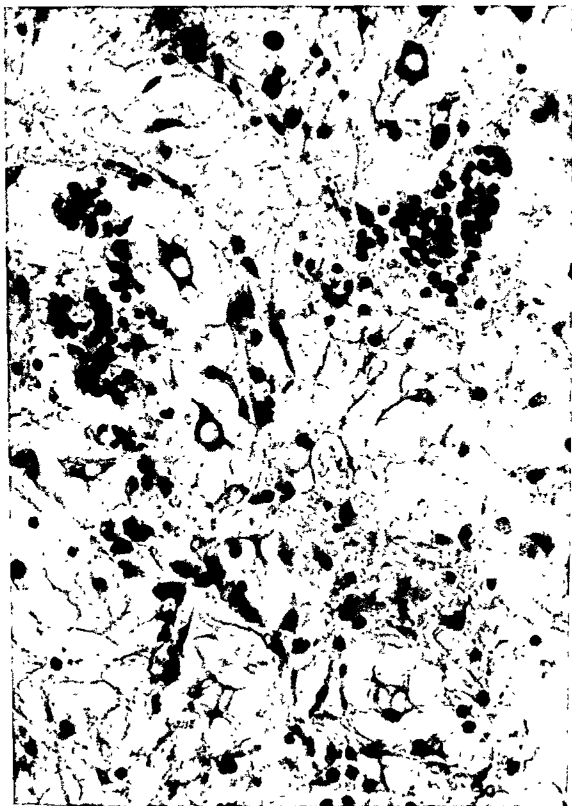


Fig 154 *Radiant Energy (Roentgen-ray) Bone Marrow Section* A patient with widespread carcinomatosis was subjected to he[illegible]

bon[illegible]

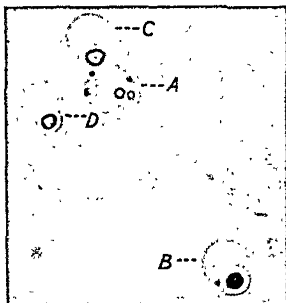
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marrow, associated with an inflammatory reaction (× 1000) *Third Panel*, Poorly cellular fibrous replacement of marrow spaces follows (× 1000) *Bottom Panel*, Osteogenic sarcoma which developed in 27 per cent of patients (× 1000) (Slides by courtesy of Dr H S Martland, Office of the Chief Medical Examiner of Essex County, Newark, N. J)

row followed, associated with an inflammatory reaction. The myeloid cavity was finally filled with poorly cellular fibrous tissue, and in 27 per cent of cases, osteogenic sarcoma developed.

Ultraviolet radiation has been shown experimentally to produce hyperplasia of megakaryocytes in the bone marrow, followed by an increase in the thrombocyte count of peripheral blood. On the basis of this observation, heliotherapy has been suggested as an adjunct in the treatment of thrombocytopenic purpura, but its value is questionable.



[illegible]

CHEMICAL

[illegible] emia, leukopenia, thrombocytopenia, or combinations thereof. *Anemia* may result from direct action of the chemical on the red blood cells (Fig 156), by its depressing the bone marrow (Figs 158 and 159), or by its creating a poorly understood refractory state associated with hyperplasia of the bone marrow (Fig. 160) In some instances, a chemical agent may react in several fashions in the same person, for example, to produce direct hemolysis and marrow aplasia, or it may affect different people quite differently (Figs. 159, 160, and 161) Fluorine is unique in occasionally causing myelophthisic anemia secondary to the osteosclerosis which results from chronic poisoning (Fig. 164) Amount and duration of exposure appear to be determining factors in the severity and type of anemia resulting from poisoning by benzol, quinacrine hydrochloride (atabrine), lead, and direct hemolysins, whereas individual idiosyncrasy seems to be more important in certain other instances

Leukopenia resulting from chemical intoxication is nearly always due to a reduction in neutrophils These may be the only cells affected, as in the case of hypersensitivity to aminopyrine (Figs. 162 and 163) or the direct action of urethane, or there may be a concomitant loss of red cells and thrombocytes following general suppression of the bone marrow. The aromatic compounds and ureides are the chief offenders in causing severe neutropenia. The nitrogen

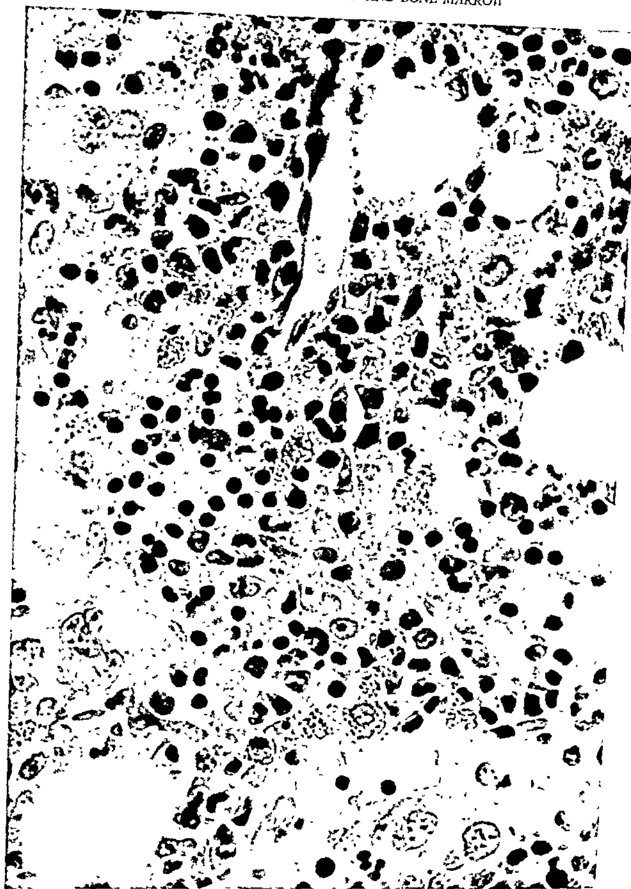


Fig. 157 *Chronic Lead Poisoning Bone Marrow Section* The patient presented a moderate degree of anemia associated with pronounced basophilic stippling of the red cells. The marrow shows marked reactive hyperplasia of red cell progenitors, the cells with darker nuclei, with normoblasts (left center) in predominance (× 1000)

Fig 158 *Neoarsphenamine Bone Marrow Section* Several weeks after beginning a second course of neoarsphenamine for the treatment of syphilis, the patient developed hemorrhagic phenomena Blood examination disclosed anemia, leukopenia, and thrombocytopenia, and the patient lived only a few weeks The marrow of all bones was virtually devoid of hematopoietic elements, and showed extensive interstitial hemorrhage. Most of the nucleated elements seen in the picture are macrophages, lymphocytes, and plasmacytes (× 500)

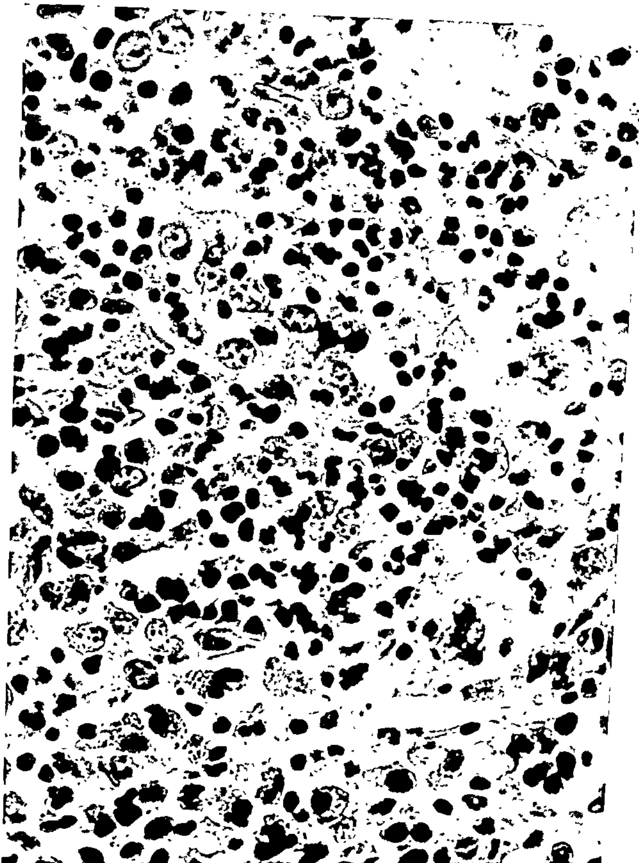
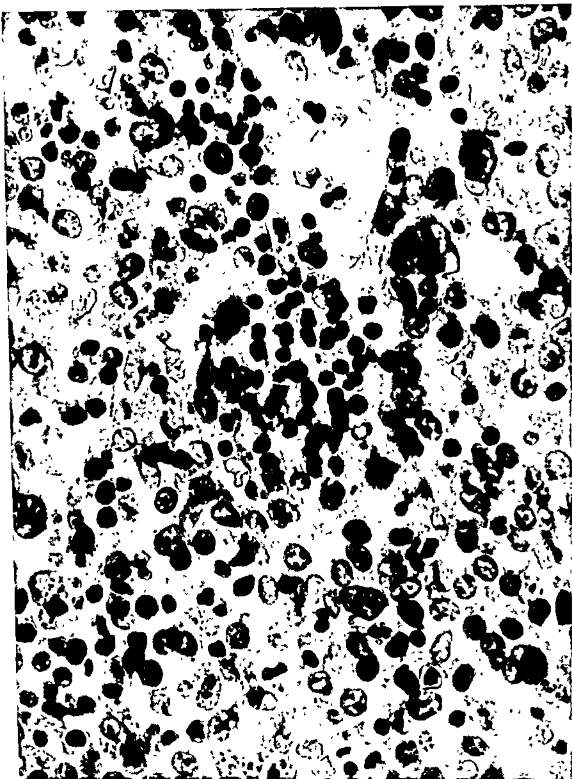


Fig 160 *Chronic Benzol Poisoning. Bone Marrow Section.* A fifty-year-old white man had been exposed to low concentrations of benzol in the atmosphere while working around "bee-hive" coke ovens over a fifteen-year period. His blood picture was characterized by normocytic, normochromic anemia, leukopenia, and thrombocytopenia, and many blood transfusions failed to raise the red cell count above 3,000,000 per cu. mm. The sternal bone marrow was totally cellular, erythroblasts and normoblasts comprising about 90 per cent in the differential count. Temporary improvement followed removal of a moderately enlarged spleen (the spleen showed reticulo-endothelial hyperplasia with marked erythrophagocytosis), but the patient lapsed into his previous state and succumbed to an intercurrent infection (× 1000)



mustards on the other hand produce a striking lymphopenia, and affect neutrophils to a lesser degree.

Thrombocytopenic purpura is generally a feature of aplastic anemia, especially in connection with poisoning by metallic or aromatic com-

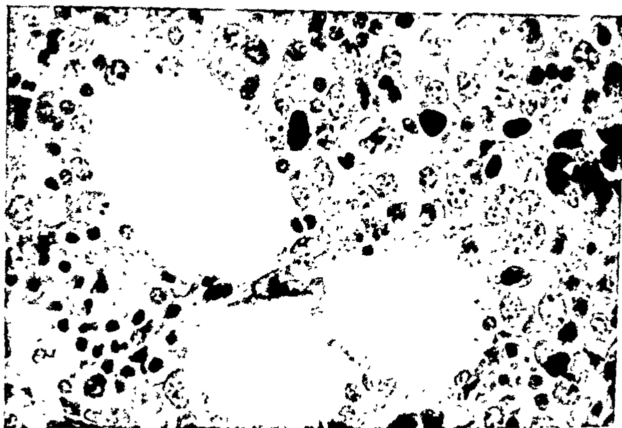


Fig. 162. *Agranulocytosis Due to Aminopyrine. Bone Marrow Section.* The patient was a thirty-one-year-old white woman who developed large gangrenous lesions of the pharynx several days after taking aminopyrine for menstrual cramps. Until death occurred ten days later, the leukocyte count ranged from 1000 to 800 per cu mm, with complete absence of neutrophils, red cell and thrombocyte counts were normal. The vertebral marrow shown here contains large numbers of myeloblasts (the large cells with vesicular nuclei), but there is no evidence of maturation in this series beyond a sparse scattering of progranulocytes. A normal island of erythropoiesis is seen in the lower left, and megakaryocytes in other fields were normal in number and appearance (× 1000).

pounds, but it may occur without significant changes in erythrocyte or leukocyte levels. In the latter instance the megakaryocytes are specifically affected and show striking degenerative changes, notably pyknosis, fragmentation, and loss of nuclei, their numbers in the marrow may be normal, increased, or decreased (Fig. 141). Sedormid, a ureide hypnotic, has produced such an effect in a good many cases.

Nonthrombocytopenic purpura that is induced by chemicals is due in some instances to idiosyncrasy, in others (especially in cases of snake venoms) to direct injury of capillary endothelium.

The majority of chemical agents known to affect the blood or blood-forming organs are listed in Table 17, along with their single or several manners of action. It is interesting to note that relatively few classes of chemicals are represented.

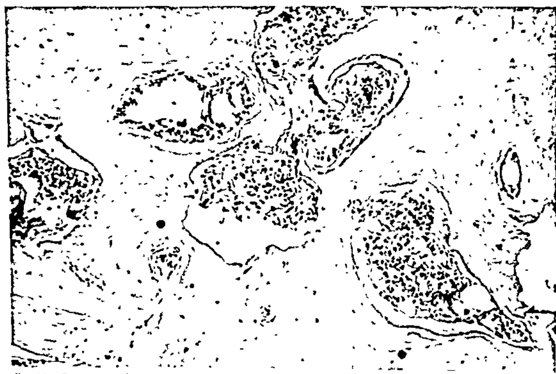


TABLE 17

SOME CHEMICAL AGENTS WHICH AFFECT BLOOD OR BLOOD-FORMING TISSUES

Agents*	*Anemia*				*Methemoglobinemia or Sulfhemoglobinemia ± Erythrocytosis*	*Leukopenia*		*Purpura*	
	HEMOLYTIC	APLASTIC	MYELOPHTHISIC	REFRACTORY		NEUTROPHILIC	LYMPHOCYTIC	THROMBOCYTOPENIC	NON-THROMBOCYTOPENIC
ALKALOIDS:									
Atropine									+
Quinine									+
AROMATIC COMPOUNDS.									
Acetanilid	+				+				
Acetophenetidin (Phenacetin)	+				+				
Aminopyrine						+			
Aniline	+				+				
Benzol (benzene)	+	+		+					
Cresol (lysol)	+								
Cymene (sulfite pulp)		+							
Dinitrobenzene	+				+				
Dinitrophenol		+				+		+	
Hair dyes (organic)				+		+		+	
Insecticides (volatile)		+							
Nitrobenzene					+				
Phenobarbital						+		+	+
Phenyl (and acetyl-phenyl) hydrazine	+								
Plasmochin (pamaquine naphthoate)	+				+				
Promin	+				+				
Quinacrine hydrochloride (atabrine)		+				+			
Salicylates								+	+
Sulfonamides	+	+		+		+		+	
Trinitrotoluene (TNT)	+	+				+			
Tripelennamine hydrochloride (pyribenzamine)						+			

* Where several types of reaction are shown opposite the same chemical, they may occur singly or in combination in a patient

TABLE 17—(*Continued*)

SOME CHEMICAL AGENTS WHICH AFFECT BLOOD OR BLOOD-FORMING TISSUES

Agents	*Anemia*				*Methemoglobinemia or Sulfhemoglobinemia ±Erythrocytosis*	*Leukopenia*		*Purpura*	
	HEMOLYTIC	APLASTIC	MYELOPHTHISIC	REFRACTORY		NEUTROPHILIC	LYMPHOCYTIC	THROMBOCYTOPENIC	NON-THROMBOCYTOPENIC
FATTY ACIDS(†)									
Lecithin	+								
Lysolecithin	+								
HALOGENS									
Fluorine			+						
Iodine									+
HYDROCARBONS, CHLORINATED									
Chloral hydrate									+
Methyl chloride	+								
METALS:									
Arsenic									
Arseniuretted hydrogen	+	+							
Arsphenamine		+						+	
Bismuth arsphenamine sulfonate (bismarsen)		+						+	
Neoarsphenamine	+	+				+		+	
Oxophenarsine hydrochloride (mapharsen)		+							
Potassium arsenite						+			
Silver arsphenamine		+						+	
Sulfarsphenamine		+						+	
Bismuth		+						+	+
Gold		+				+		+	
Lead	+								
Mercury		+							+
Silver	+	+							
MUSTARDS									
Mustard gas		+							
Nitrogen mustards							+	+	
Onion juice	+								

† Fatty acids in general appear to be hemolytic agents, high fat diets are accompanied by increased excretion of urobilinogen

TABLE 17—(*Continued*)

SOME CHEMICAL AGENTS WHICH AFFECT BLOOD OR BLOOD-FORMING TISSUES

Agents	*Anemia*				*Methemoglobinemia or Sulfhemoglobinemia ±Erythrocytosis*	*Leukopenia*		*Purpura*	
	HEMOLYTIC	APLASTIC	MYELOPHTHISIC	REFRACTORY		NEUTROPHILIC	LYMPHOCYTIC	THROMBOCYTOPENIC	NON-THROMBOCYTOPENIC
STEROIDS:									
Estradiol benzoate		+							
Saponin (sapotoxin)	+								
UREIDES:									
Aprobarbital (alurate)						+		+	
Hydantoin (mesantoin)		+							
Sedormid								+	
Thiouracil						+			
Thiourea						+		+	
Trimethadione (tridione)		+							
Urethane						+			
VEGETABLE MATTER:									
Castor bean (ricin)	+								
Fava bean	+								
Pollens	+								
VENOMS (via lecithinase).	+								

XIII

LEUKOCYTOSIS, LEUKEMOID REACTIONS, AND LEUKOPENIA

The total and differential leukocyte count constitutes one of the most valuable diagnostic aids that the clinical laboratory has to offer, as well as affording a medium by which the reaction of the patient to a noxious agent can be followed. This presupposes that the total count has been carefully performed, the blood film well stained, the cells accurately identified, and an adequate number of cells tallied to be representative of the overall leukocyte picture.

Leukocytosis refers to a total leukocyte count above the generally accepted normal high of from 10,000 to 11,000 per cu. mm. Levels of 50,000 or above in patients who do not have leukemia are spoken of as *leukemoid blood pictures*. Leukemoid reactions also include lower counts, even below normal, in which considerable numbers of immature white blood cells appear in the peripheral blood. Total counts under 4000 per cu. mm. are called *leukopenic*.

KINDS OF REACTION

Changes in the leukocyte picture may involve any or all types of white cells. Certain kinds of infection characteristically evoke a particular sort of leukocytic reaction. Thus, pyogenic organisms usually call forth a neutrophilic leukocytosis, whereas there is apt to be neutropenia with actual or relative lymphocytosis in many virus infections Exceptions are frequently observed, however, so that only coarse trends can be indicated (Table 18).

Tuberculosis affords a good example of the variety of leukocyte pictures that have been noted in a single disease, viz, neutropenia to myeloleukemoid reaction, lymphopenia to lympholeukemoid reaction, eosinophilia, and monocytosis Within this wide range of leukocyte response, certain clinicopathologic correlations have been possible. Cases of acute miliary tuberculosis usually show leukopenia with a relatively high percentage of neutrophils, rarely a leukemoid reaction. Moderate neutrophilic leukocytosis is generally associated with extending ulceration, involvement of serous surfaces, or secondary infection. Most cases of leukemoid reaction have occurred in patients with extensive lymphatic tuberculosis. The ratio between lymphocytes and monocytes has prognostic significance; if the ratio of monocytes to lymphocytes exceeds 1:3, the process is probably progressive, but if less than 1:3, the outlook is better. In short, in tuberculosis the neutrophil indicates tissue destruction or suppuration, the monocyte indicates new tubercle formation, while the lymphocyte indicates healing. Eosinophilia is likely an evidence of allergic response in some persons to bacteria or products of bacterial metabolism.

Immaturity of circulating leukocytes is confined largely to the granulocytic series, although prolymphocytes are frequently encountered in the leukocytoses of children. Attention has been given chiefly to neutrophils, and a number of methods for classifying them have been devised, as well as formulas and indexes to assist in interpreting the findings. Most of these can be discarded without mention.

The more frequently used classifications are tabulated as follows:

Neutrophil Type	*Norm (%)*	*Schilling*	*Farley and others*
Myeloblast			
Progranulocyte		Myelocyte	
Myelocyte			Nonfilamented Forms
Metamyelocyte		Juvenile	
Band (Stab) Form	3–5	Stab Form	
Segmented (Filamented) Form	54–62	Segmented Form	Filamented Form

My own preference is to use the filament-non-filament count on blood films where a scan at low magnification discloses little or no immaturity apart from metamyelocytes (juveniles) and band cells. If immaturity exceeds this, the cells should be tallied precisely according to the *first column*. The appearance of young cells of the granulocytic series in the peripheral blood is referred to as a "shift to the left," and the finding of large neutrophils with hypersegmented nuclei (5 or more lobes) as a "shift to the right."

Qualitative changes in neutrophils have considerable significance. Toxic granulation is a feature of severe infections and other toxic states, characterized by blue to blue-black granules of varying size in the cytoplasm; small ones are generally admixed with normal pink granules, large ones being associated with few or no pink granules. The "toxic granules" are not peroxidase positive. Care must be exercised that diffuse blue staining of neutrophil granules owing to improperly prepared buffer solution is not interpreted as toxic granulation. Other degenerative changes in neutrophils comprise nuclear pyknosis, and vacuolization of cytoplasm and nuclei (Figs. 168, 170, and 186)

Neutrophilic leukocytosis almost always results from hyperplasia of progenitor cells in the bone marrow. Thus, the blood picture is merely a reflection of the ability of the marrow to respond to a need for neutrophils elsewhere in the body. A shift to the left in the peripheral blood means that cells are being liberated from the marrow at such a rate that the normal maturation process is not completed, compelled by an unusual demand for this type of cell; in these cases there is a relative and actual increase of myeloblasts and progranulocytes in the marrow, as well as a decrease in the erythro-granulocytic ratio. Red cell precursors and megakaryocytes share the hyperplasia, however, if to a lesser degree. Generalized hyperplasia of the bone marrow under these circumstances is evidenced by the occasional appearance of nucleated red cells and megakaryocytes in the peripheral blood; sections of various tissues at autopsy show megakaryocytes swept from the hyperplastic marrow and lodged in the capillary beds.

In some instances, there has been no time for a hyperplastic reaction, and the marrow has been suddenly emptied of neutrophils (see Figs. 149 and 150) resulting from a sudden call for these cells, with death ensuing soon there-after.

Eosinophilic leukocytosis is reflected by an increase in these specific myelocytes in the marrow (see Figs. 166 and 167). Lymphocytosis and monocytosis seldom affect the marrow to any extent.

LEUKEMOID REACTIONS

In *leukemoid states*, the blood picture more or less closely resembles that of true leukemia, although the generalized tissue reactions of leukemia are not present. When anemia, hemorrhagic phenomena, splenomegaly, or lymph node enlargement are associated, it may be difficult or indeed impossible to rule out leukemia.

Leukemoid blood pictures may be either myeloid or lymphoid in type. They may be classed as leukemoid either by the magnitude of the leukocytic reaction without immaturity (hyperleukocytosis) (Fig 186) or by reason of immature or atypical cells in the circulating blood (Fig. 168) irrespective of the total leukocyte count. Nucleated red blood cells frequently spill into the peripheral blood, especially when anemia coexists, owing to hemorrhage or hemolysis.

TABLE 18

Causes and Types of Leukocytosis

*Frequent Causes of Leukocytosis**	*Neutro-philia*	*Eosino-philia*	*Baso-philia*	*Lympho-cytosis*	*Mono-cytosis*	*Plasma-cytosis*
ALLERGY		+				Occ.
ANOMALY, FAMILIAL		+				
DEHYDRATION	+	+	+	+	+	
ERYTHREMIA	+	+	+			
HEMOLYSIS	+					
HEMORRHAGE	+					
INFECTIONS: Bacilli in general				+		
Also Brucellosis				+	+	
Diphtheria	+					
Escherichia coli infections	+					
Gas gangrene	+					
Plague	+					
Tuberculosis	+	+		+	+	
Tularemia	+			+		
Typhoid fever				+	Occ.	
Cocci in general	+					
Also Chorea	+	+				
Gonorrhea	+	+				
Scarlet fever	+	+				
Subacute bacterial endocarditis	+				+	
Fungi: Initially	+					
Subsequently				+	+	
Parasites		+				
Protozoa	+				+	
Rickettsia	+			+	+	
Spirochetes	+	+		+		
"Tropical eosinophilia"		+				

* This tabulation does not imply that the leukocytosis indicated necessarily occurs in every case, but is intended merely to indicate a trend. Indeed, severe infections and chemical intoxications even more frequently induce leukopenia.

Where two or more types of leukocytic response are tallied under a given condition, they may occur alone or in combination in a patient.

TABLE 18—*Continued*

Frequent Causes of Leukocytosis	*Neutrophilia*	*Eosinophilia*	*Basophilia*	*Lymphocytosis*	*Monocytosis*	*Plasmacytosis*
VIRUSES, especially infectious mononucleosis and lymphocytosis			Occ.	+		Occ
IRRADIATION OF TUMORS	+	+				
PHYSIOLOGIC: Newborn	+					
Infancy and childhood				+		
Accelerated blood flow (emotion, exercise, etc.)	+					
SKIN DISEASES, *especially pemphigus* and dermatitis herpetiformis		+				
TISSUE DAMAGE: Burns	+					
Infarcts	+					
Surgical procedures and other direct trauma	+					
TOXEMIAS. Endogenous: Diabetic acidosis	+					
Eclampsia	+					
Gout	+					
Uremia	+					
Exogenous. Acetanilid	+					
Arsenicals	+					
Benzol compounds	+					
Camphor	+	+				
Carbon monoxide	+					
Copper sulfate		+				
Digitalis	+					
Epinephrine	+					
Lead	+					
Phenacetin	+					
Phosphorus		+				
Pilocarpine		+				
Pyridine	+					
Pyrogallol	+					

TABLE 18—*Continued*

Frequent Causes of Leukocytosis	*Neutrophilia*	*Eosinophilia*	*Basophilia*	*Lymphocytosis*	*Monocytosis*	*Plasmacytosis*
Turpentine	+					
Tetrachlorethane					+	
Venoms	+					
TUMORS Hodgkin's granuloma	+	+			+	
Tumors involving bone marrow	+	+				
Tumors involving gastro-intestinal tract or liver	+					
Tumors involving serous surfaces	+	+				
Ovarian tumors		+				
Rapidly growing tumors	+					
MISCELLANEOUS CONDITIONS Loeffler's syndrome		+				
Periarteritis nodosa		+				
Pernicious anemia		+				
So-called "storage diseases"					+	

Causes of Leukemoid Reactions. The more frequent causes of leukemoid reactions are listed in Table 19.

Congenital Cell Defect. Pelger's anomaly of granulocytes (congenital cell defect) is a familial trait characterized by the failure of the nucleus of these cells to segment beyond two lobes (Fig 165) Many band cells are present, along with a scattering of metamyelocytes and myelocytes. Nuclear chromatin is coarse and lumpy. The finding of this cellular atypism in an apparently healthy person has on a few occasions been interpreted as indicating a subclinical phase of chronic myelogenous leukemia. It is well for these people to know of their anomaly and to tell their physician when a blood count is taken.

Hemoclastic Crisis The bone marrow reaction following sudden hemoclastic crises frequently floods the peripheral blood with neutrophilic leukocytes and their precursors (Figs. 122 and 124). I recall a case of familial hemolytic jaundice erroneously diagnosed leukemia for this reason; to make the situation still more difficult, the abnormal erythrocyte fragility had been masked by extremely high levels of the more resistant reticulocytes.

Hemorrhage. Severe hemorrhage is sometimes followed by neutrophilic leukocytosis, occasionally attaining heights of from 50,000 to 75,000 per cu. mm as a result of rapid and diffuse bone marrow hyperplasia.

Hodgkin's Disease. The granulomatous form of Hodgkin's disease often presents a leukemoid reaction, usually not very striking, but a few patients have had leukocyte counts of from 100,000 to 250,000 per cu mm. Eosinophils may be prominent and are occasionally the only proliferating cell type (Figs. 166 and 167).

Fig. 165. *Congenital Cell Defect* (Pelger's Anomaly of Granulocytes). *Blood Film.* Both neutrophils and eosinophils (left center) are bilobate, some filamented, others corresponding to band cells or metamyelocyte forms, a few neutrophils with round nuclei were noted in other fields This person was quite well, and other members of his family presented the same blood picture (× 650).

types, although an occasional myelocyte (lower right) was encountered (× 1280).

Fig 167. *Eosinophilic Leukemoid Reaction (Hodgkin's Disease) Bone Marrow Section* Marrow from the case pictured in Fig 166 showed general hyperactivity, with particular prominence of eosinophilic myelocytes The disproportion between cells of the granulocytic series and red cell progenitors (cells with dark compact nuclei) is not as marked as one generally finds in eosinophilic leukemia (× 1000)

Fig 168 *Neutrophilic Leukemoid Reaction Blood Film.* This patient had a tantalum plate inserted in a skull wound and developed gas gangrene of the operative field The following day, the leukocyte count was over 60,000 per cu mm, with many myelocytes appearing in the film (× 2280)

Infections. (Discussed in detail in Chapter 14.) Infections by a variety of viruses, bacteria, and parasites have produced leukocyte counts ranging from 50,000 to 175,000 per cu. mm., with varying degrees of immaturity of the cells, occasionally simulating acute myeloblastic leukemia. Tuberculosis has received much attention in the literature, and is rather unique in having given rise to both myeloid and lymphoid types of blood picture; patients with extensive involvement of lymph nodes and spleen (occasionally with acutely disseminated miliary tuberculosis) are most apt to develop a leukemoid reaction. Of the pyogenic organisms the pneumococcus, meningococcus, and streptococcus have been responsible for hyperleukocytosis in most instances. Gas gangrene (Figs. 168, 169, and 170) may also cause a leukemoid reaction. Leukemoid states in children are generally caused by chickenpox and whooping cough (Figs. 171 and 172), being uniformly lymphoid in type I have observed a few small children with striking lymphocytosis associated with lowgrade infections of the pharynx and middle ear. Several cases of congenital syphilis with counts exceeding 100,000 per cu. mm. have been described. Infectious mononucleosis (Figs. 176, 177, 178, 179, 180, 181) and lymphocytosis (Figs 174, 175) seldom offer a serious problem in differentiation from leukemia, although the leukocyte counts are occasionally very high. Eosinophilic leukocytosis in connection with parasitism (Fig. 211) and so-called "tropical eosinophilia" (Fig. 173) sometimes reaches levels suggestive of eosinophilic leukemia

Toxemias. Extensive burns have provoked marked neutrocytosis with young forms in the peripheral blood (Fig. 149); the marrow in some instances is depleted (Fig. 150), in others hyperplastic. One case of *eclampsia* is reported to have had a white cell count of 100,000 per cu mm., and a patient with *mercury poisoning* reached a level of 69,500 with 24 per cent myelocytes and a clinical picture resembling granulocytic leukemia.

Tumors. A leukemoid blood picture, usually associated with large numbers of nucleated red blood cells (Fig. 94) is apt to result from tumors that extensively displace bone marrow. A few cases of metastasis to the spleen without bone involvement have behaved likewise. The leukocytosis is generally neutrophilic, but lymphocytic and eosinophilic responses have been described.

Differential Diagnosis The differential diagnosis from granulocytic leukemia is usually possible by examination of the bone marrow, most cases showing a nonspecific type of hyperplasia in which the earlier stages of granulocytes are not particularly conspicuous (Fig. 169). The myelosclerotic variant of granulocytic leukemia has already been described (p. 84), as well as the widespread marrow displacement by osteoplastic and desmoplastic types of tumors (see Fig. 94). Eosinophilic reactions simulating leukemia can usually be separated by qualitative characters of the circulating eosinophils. Eosinophils of leukemia generally show nuclear maturity; i.e., segmentation, but granularity of the cytoplasm is imperfect, and the cytoplasm proper is frequently more deeply basophilic than normal. Ring nuclei are often encountered in eosinophilic leukemia (Fig. 173).

It is still easier to rule out lymphocytic leukemia, if one has a generous sample of bone marrow for study, but it must be remembered that marrow involvement in the chronic form of this condition is patchy, and it is possible to aspirate normal or hyperplastic tissue from unaffected areas. Again, small nodules of lymphocytes that occur occasionally in the marrow for no apparent reason are indistinguishable from early lesions of lymphocytic leukemia. These facts serve warning against offering an irrevocable diagnosis and prognosis without final proof.

Clinical features of the syndrome under consideration may serve either to clarify or confuse the issue. One should not overlook the cases of true leukemia which are not necessarily featured by a palpable spleen or enlarged lymph nodes, and in the absence of these signs, a questionable blood picture had best not be dismissed as leukemoid without further thought.

In a certain number of cases, time is the only diagnostician. The patient either gets well or dies, and if he dies, the autopsy alone will disclose the true nature of the condition.









Fig 172. *Lympholeukemoid Reaction (Pertussis) Blood* The patient was a nine-year-old girl who had the usual manifestations of severe whooping cough. The total leukocyte count was 84,000 per cu. mm , and nearly all cells in the [illegible]



Fig [illegible] from the sternum of the patient [illegible] seen in [illegible] present elsewhere in the smear, were attributed to dilution with peripheral blood, as sectioned fragments of aspirated marrow failed to disclose lymphocytic foci in the tissue proper (- 2280)

LEUKOPENIA

Leukopenia occurs consistently in some conditions, occasionally in others that have been listed as being characterized by leukocytosis, and as an early or late phase in still others. The major causes of leukopenia (chiefly neutropenia) are listed in Table 20.

In most of the conditions itemized in Table 20, the blood and marrow pictures have been described in other chapters. Study of the bone marrow is usually helpful, often diagnostic. One may find a hyperplastic, displaced, normal, or empty marrow. With a relatively full marrow, it is easy to sort out the aleukemic leukemias, pernicious anemia, and sprue, others show a rather nonspecific type of hyperplasia. Reference to the chapter on marrow displacement shows a specificity of the marrow picture in some instances. Insignificant alterations in appearance of the marrow are found in the allergic states, Banti's and Felty's syndromes, and infectious mononucleosis. Among the infections, organisms can usually be found in the marrow in kala-azar and malaria, or in the blood (malaria, relapsing fever). Tuberculosis and typhoid fever often produce lesions that can be identified in marrow sections; a case of profound leukopenia was diagnosed as typhoid fever by sternal biopsy (Figs. 188 and 189), and the diagnosis confirmed by bacteriologic examinations. Recently a so-called "lupus erythematosus cell" (L E. cell) has been described (Fig. 192), apparently being a segmented neutrophil which has engulfed a large round basophilic body, possibly a "round cell" nucleus; they are not constantly found in the marrow in this disease, but when present are probably of some diagnostic significance. Patients with empty marrows have the associated reduction in red cells and thrombocytes typical of aplastic anemia; in such instances it remains to determine the offending agent, if possible.

TABLE 20

CAUSES OF LEUKOPENIA

Allergy
- Anaphylactoid shock
- Foreign-protein reaction (early)

Cachexia

Chemical agents
- Those producing aplastic anemia
- Those specific for neutrophils (maturation defect?)
- Those with particular destructive effect on lymphocytes

(See Table 17.)

Disorders of the hemolytopoietic system
- Aleukemic leukemia
- Aplastic anemia, idiopathic
- Banti's syndrome
- Felty's syndrome
- Liver disease, advanced
- Myelophthisic states
- Panhypersplenism
- Pernicious anemia, relapse
- Primary splenic neutropenia
- Sprue

Infections
- Any overwhelming one
- Bacterial
  - Brucellosis
  - Tuberculosis, some cases
  - Typhoid fever and allied salmonella infections
- Protozoal
  - Kala-azar
  - Malaria
- Spirochetal
  - Relapsing fever (interval period)
- Virus
  - Dengue fever
  - Infectious mononucleosis, occasional case
  - Influenza
  - Measles
  - Pappataci fever
  - Psittacosis
  - Rubella
  - Smallpox, early

Irradiation

Unknown cause
- Disseminated lupus erythematosus

XIV

INFECTIONS

The previous chapter included a general consideration of leukocyte responses to various types of infection. The purpose of this section is to mention a little more specifically the reactions in the blood and blood-forming organs following infection, and to single out certain conditions in which the infectious agent can be directly identified by examination of the blood or bone marrow. The term "infection" is used in the broad sense to include invasion of the body by viruses, rickettsias, spirochetes, bacteria, fungi, protozoa, and helminths

The *hypochromic anemia* that one observes with fair regularity in association with infections, particularly protracted ones, is often more severe than the blood count would indicate, because of the concomitant reduction in blood volume It will not respond to the administration of iron as does the anemia of iron deficiency or chronic hemorrhage. While the intake and absorption of iron may be quite adequate, plasma iron remains low; even iron given by vein is soon followed by a reduction in plasma iron to preinjection levels. The studies of Wintrobe and co-workers, using "tagged iron" (Fe^{59}), have indicated that "the major diversion of plasma iron in infection is to the ordinary storage tissues, mainly the liver," and that "this diversion is related to the hypoferremia." They also showed that a similar situation could be brought about experimentally by the production of sterile abscesses, so that the *anemia of infection* should probably be termed the *anemia of inflammation.*

Other conditions related to infection may also be present in the production of anemia, as will be shown in the following paragraphs. For example, certain bacteria (clostridia and others) and protozoa (plasmodia) are directly hemolytic, while certain of the helminths (ancylostoma, diphyllobothrium) cause severe anemia only in certain persons, rendered susceptible perhaps by nutritional deficiencies

VIRUS DISEASES

Viruses are particulate bodies which pass through filters that retain bacteria. They range in size from 10 to 275 millimicrons, and only the larger ones (vaccinia, psittacosis-lymphogranuloma group) are visible by the usual microscopic technics, although most have been pictured on electron micrographs. Viruses require the presence of living cells for growth. Some produce "inclusion bodies" in the nuclei or cytoplasm of host cells (probably combinations of virus and cell product), while larger viruses form "elementary bodies" in the cytoplasm (probably the virus particles proper). Virus diseases may be transmitted by inhalation or ingestion of infectious material, by direct contact with an infected person, or by insect vectors, varying with the type of virus. The diagnosis depends on identification of the virus by inoculation of susceptible animals or, preferably, by demonstration of specific antibodies in the patient (neutralization or complement-fixation tests).

In the two diseases showing the most striking blood pictures, infectious lymphocytosis and infectious mononucleosis, a virus has not been definitely identified, they are classed in this group, nevertheless, because their infectiousness is unquestionable, yet no visible agent has been demonstrated.

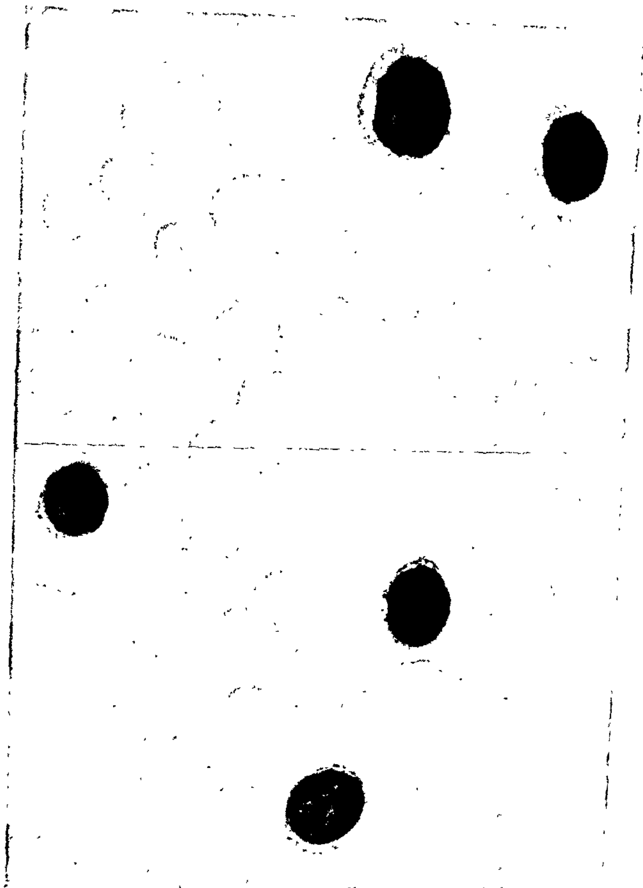


Fig 174 *Infectious Lymphocytosis Blood* In October 1944 there was an epidemic of infectious lymphocytosis at the Children's Heart Hospital in Philadelphia, evident chiefly by routine blood counts, as clinical manifestations of the disease were virtually nonexistent. *This patient had a leukocyte count of 51,900 per cu mm.,* 44,600 of which were well-differentiated lymphocytes, 6700 neutrophils, and 520 eosinophils There was no enlargement of the spleen or lymph nodes, and the heterophil antibody reaction was negative (slide by courtesy of Doctors Ella Roberts and John Bauer) (× 2280)

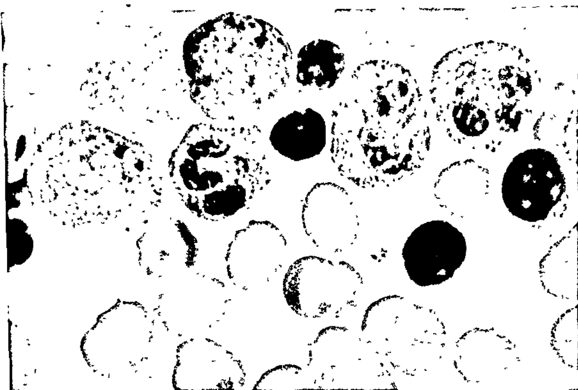


Fig 175 *Infectious Lymphocytosis. Bone Marrow Smear.* (Same case as Fig. 174) Lymphocytes are present in no greater abundance than one would anticipate from dilution with peripheral blood (slide by courtesy of Doctors Ella Roberts and John Bauer) (× 2280)

Infectious Lymphocytosis

This disease was recognized as an entity by Smith * He described it as an infectious and contagious disease, occurring mostly in children, with a probable incubation period of twelve to twenty-one days. Clinical manifestations are either mild or absent, the striking feature being hyperleukocytosis (frequently over 50,000 and occasionally reaching 100,000 per cu mm) with a relative and absolute increase in small lymphocytes of normal appearance (Fig 174). The lymphocytosis lasts for three to five weeks, sometimes longer. Aspirated bone marrow has been reported to contain an increased number of lymphocytes, representing as much as 90 per cent of nucleated cells in some instances. This is not in agreement with my findings in a relatively few cases, where the lymphocytosis in marrow smears was attributed to dilution with peripheral blood, and the marrow proper was regarded as normal (Fig 175) I have not had marrow tissue for section to confirm this impression. Thus far, there has been no reported fatality from infectious lymphocytosis.

* Am J Dis Child, *62* 231, 1941

Infectious Mononucleosis

This disease occurs sporadically or in epidemics with children or young adults chiefly affected, although no age group is immune. Contagiousness is apparently low. The incubation period seems to be about ten days to two weeks, and the onset is marked by fever, sore throat, and enlarged, tender lymph nodes (especially cervical) in the typical case The clinical manifestations are exceedingly diverse, however. While the lymphatic structures of the body are primarily and uniformly affected, virtually all tissues may be involved more or less extensively, † and symptoms depend largely on the organs or systems implicated to any marked degree. An analysis of the admission diagnoses of Read and Helwig's 300 patients ‡ serves to illustrate the confusing clinical pictures frequently observed:

† Custer and Smith: Blood, *3* 830, 1948

‡ Arch Int. Med, *75* 376, 1945.

Admission Diagnoses

Hematopoietic System	Infectious mononucleosis	37
	Lymphadenitis	10
Respiratory System:	Epistaxis	4
	Acute sinusitis	12
	Nasopharyngitis	45
	Acute pharyngitis	64
	Acute tonsillitis	36
	Bronchitis	10
	Atypical pneumonia	19
	Influenza	4
Digestive System.	Vincent's angina	5
	Gastro-enteritis	3
	Jaundice	6
Nervous System.	Psychoneurosis	1
	Suspected meningitis	3
	Heat exhaustion	2
Miscellaneous.	Malaria	7
	Reaction to typhoid vaccine	1
	Cervical mass	2
	Cervical myositis	5
	Diagnosis uncertain	12

Admission Complaints

Hematopoietic System	Swollen lymph nodes	81
Respiratory System.	Epistaxis	6
	Sore throat	146
	Cough	38
Digestive System	Anorexia	58
	Nausea	9
	Vomiting	7
	Abdominal pain	14
	Jaundice	6
Nervous System	Headache	71
	Vertigo	9
Miscellaneous	Malaise	72
	Fever	67
	Arthralgia	7
	Myalgia	5
	Cutaneous eruption	11

Positive Signs

Hematopoietic System	Generalized lymphadenopathy	172
	Cervical lymphadenopathy	123
	Tender lymphadenopathy	67
	Palpable spleen	104
	Tender spleen	39
	Petechiae	9
	Oral cavity	4
	Generalized	9
Respiratory System	Epistaxis	6
	Follicular pharyngitis	112
	Membranous pharyngitis	34
	Acute tonsillitis	29
	Peritonsillar abscess	7
	Hemoptysis	3
Digestive System:	Gingivitis	37
	Vomiting	7
	Diarrhea	4
	Palpable liver	47
	Jaundice	11
	Abdominal tenderness	3
Nervous System.	Stiff neck	2
	Mild stupor	3
	Delirium	1
Miscellaneous:	Myositis	5
	Dermatitis	16

Even so, this tabulation does not begin to cover the entire range of signs and symptoms that have been described in isolated cases. Consequently, reliance must be placed on the more positive findings in the blood, and bone marrow study is occasionally required to rule out leukemia.

Examination of Blood. Leukocytosis is usually present, although normal or diminished counts may be found. Under any circumstances there is an increase in mononuclear cells (usually over 60 per cent of the total), some of which are ordinary lymphocytes and monocytes. The characteristic cells are atypical lymphocytes of varying size which fit roughly into three groups described by Downey and McKinlay.* Cells of *Type I* (Fig. 176) are most commonly observed. Their nuclei are usually eccentrically placed and may be ovoid, but frequently have one or more indentations of varying depth and are sometimes lobulated, the chromatin network is coarse and not sharply defined from the parachromatin. The cytoplasm of most cells contains flaky dark blue spongioplasm imparting a foamy or mottled appearance; it is often concentrated around the periphery, while the cytoplasm adjacent to the nuclear indentation may have a pinkish cast, and fine carmine granules of the lymphocyte type are frequently present. The cells react negatively with the peroxidase technic. Other cells in this group contain much less basophilic substance in the cytoplasm (Fig. 177).

Cells of *Type II* present more regular nuclei with very coarse chromatin strands distinct from the parachromatin, resembling in some measure plasmacyte nuclei; the cytoplasm is somewhat smoother than that of Type I cells

* Arch Int Med, *32* 82, 1923




Fig 176 *Infectious Mononucleosis. Blood* Typical of Downey's Type I abnormal lymphocyte, these cells have irregularly indented nuclei and flaky basophilia most pronounced in the periphery of the cytoplasm (× 2100).




Fig. 177. *Infectious Mononucleosis Blood.* Variant, of Downey's Type I atypical lymphocyte, larger cells with indented nuclei, lighter blue-gray cytoplasm, and fine carmine cytoplasmic granules (× 2100).

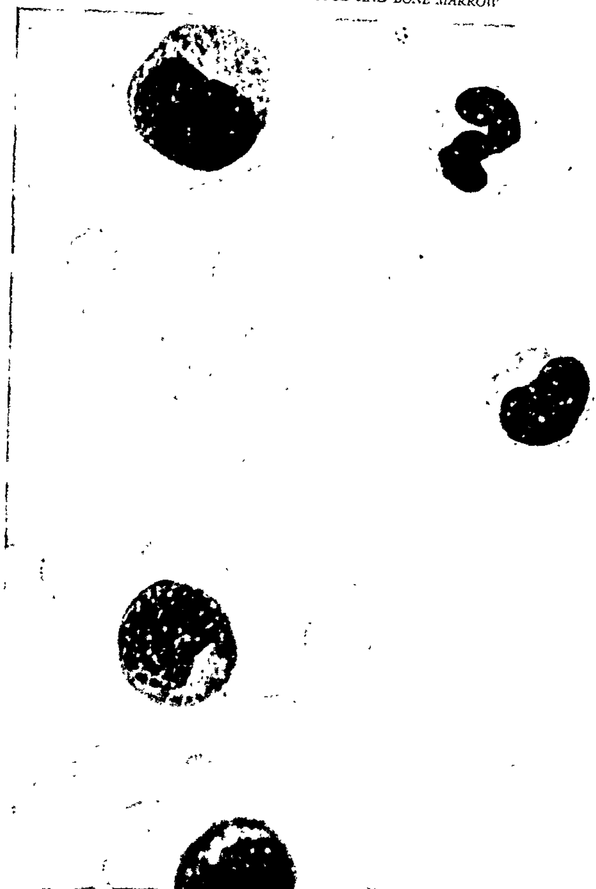


Fig. 178. *Infectious Mononucleosis Blood.* Atypical lymphocytes correspond roughly to Downey's Type II. They are large, with ovoid or only slightly indented nuclei which have coarse chromatin in sievelike arrangement. The cytoplasm has fewer vacuoles and is less spongy than that of Type I, and is frequently less basophilic (× 2250)

(Fig. 178). *Type III* cells (Fig. 179) may be mistaken for abnormal cells of lymphocytic leukemia, their nuclei are apt to be disproportionately large, and the more delicate chromatin is arranged in a fine skein. The cytoplasm is moderately to deeply basophilic. Vacuoles appear frequently in both nucleus and cytoplasm. Nucleoli have been described, but I have never been satisfied that a true nucleolus occurs in the abnormal lymphocytes of infectious mononucleosis. Stress has been laid by some authors on "fenestration" (vacuolization) as an aid in the recognition of mononucleosis cells; the presence of vacuoles is not helpful, as they occur both in normal and leukemic lymphocytes.

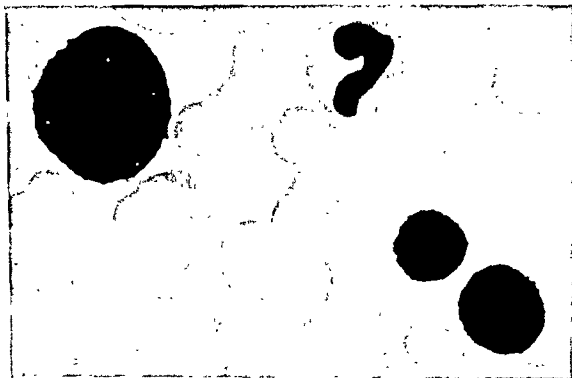


Fig. 179 [illegible]

The leukocyte and temperature curves usually parallel one another, so that the white count is normal or nearly so when the convalescent period is reached. Atypical lymphocytes are occasionally found in the blood smears for some time after the patient is quite well; one of our interns did not have a completely normal blood picture until thirteen months after the onset of his illness, and his heterophil antibody titer remained elevated for nearly three months.

Neutrophils may be increased during the first few days of the disease and occasionally persist. A well-marked neutrophilic leukocytosis appearing during the acute phase generally signifies a complication of some sort; for example, in one of our patients whose spleen ruptured spontaneously, neutrocytosis virtually masked the blood picture of the primary disease.* Other patients have shown a striking reduction in absolute numbers of circulating granulocytes, but this is unusual.

Anemia is rarely observed in infectious mononucleosis as part of the disease I have studied two cases and know of one other in which acute hemolytic anemia coincided with the usual manifestations of mononucleosis. Sple-

* Smith and Custer: Blood, *1* 317, 1946

nectomy was performed in one of our cases, *not in the other;* both patients made an uneventful recovery.

Thrombocytopenic purpura has been reported in sufficient number of cases that it appears to be a feature of the disease, although a rare one. A prolonged bleeding time is not unusual, however, even though the thrombocyte count is normal, probably due to capillary damage; the coagulation time is invariably normal.

Examination of Bone Marrow. On several occasions the bone marrow has been reported to contain large numbers of atypical lymphocytes. This has been flatly disproved. The marrow is generally normal or moderately hyperplastic. Hyperplasia for the most part is limited to granulocytes and megakaryocytes, with neutrophil progenitors occasionally showing degenerative changes. Erythropoietic hyperplasia is seldom seen (Fig. 180). In one patient with thrombocytopenic purpura, the megakaryocytes were described as showing greatly diminished thrombocyte production.* Any lymphocytosis that may be observed in marrow aspirates can be accounted for by dilution with peripheral blood.

Diagnosis. The Paul-Bunnell test for *heterophil antibodies* has proved a reliable diagnostic criterion in over 90 per cent of cases, if the so-called "Forssman antibodies" have been ruled out by appropriate differential absorptive agents. It is difficult to fix an exact borderline, but titers over 1:80 are generally significant. The heterophil antibody titer is not an index of the severity of the disease or the degree of leukocytic reaction; striking variations in the curves are seen from patient to patient (Fig 181). False positive tests for syphilis occur in 15 to 20 per cent of cases, occasionally lasting for several months, and agglutinins for *Salmonella typhosa* and other bacteria have also been demonstrated.

The *prognosis* is good, although many patients become extremely ill. Rupture of the spleen has been one of the more serious complications and has been responsible for a few deaths † Infectious mononucleosis is one of the causes of the Guillain-Barré syndrome from which several fatalities have resulted. The cause of death in isolated cases has been reported as myocarditis, pneumonia, edema of the larynx, and hemorrhage from a deep pharyngeal ulcer.

Other Virus Diseases

During the incubation period, *measles* usually causes a slight neutrophilic leukocytosis with a marked left shift; on the appearance of the rash, there is a reduction in both neutrophils and lymphocytes, and the total leukocyte count may reach 2500 to 3000 cells per cu. mm. The count remains low for a week or so in most cases, then rises slowly to normal levels; a few plasmacytes are generally present Hemorrhagic phenomena are sometimes seen, but the thrombocyte count is normal. The blood picture in *German measles* (rubella) differs in that there is neutropenia and lymphopenia from the outset. By the fifth day, lymphocytes have increased to exceed normal levels, followed by an actual lymphocytic leukocytosis during the next few days. Plasmacytes appear regularly early in the disease, and may exceed 10 per cent of the total count; prolymphocytes are sometimes numerous.

Mumps is characterized by neutropenia, with relative or absolute lymphocytosis and frequently monocytosis. Development of orchitis usually causes neutrophilic leukocytosis.

The initial phases of *smallpox* (variola) are marked by a minor neutrophilic leukocytosis, followed by a period of neutropenia during the maculopapular stage. With the development of pustules, there is a second rise in neutrophils which may reach fairly high levels; in some cases lymphocytes and monocytes participate rather extensively in the secondary leukocytosis. It is common for hypochromic anemia to appear, especially in the hemorrhagic form of the disease; hemorrhagic manifestations are not associated with thrombocytopenia. *Chickenpox* (varicella) induces a slight neutrophilic leukocytosis at first, which is soon followed by lymphocytosis that occasionally reaches leukemoid proportions. Eosinophilia is often noted in the recovery phase.

Mild cases of *influenza* may present either a normal or slightly reduced leukocyte count.

* Dameshek and Grassi *Blood, 1* 339, 1946

† Smith and Custer *Blood, 1* 317, 1946

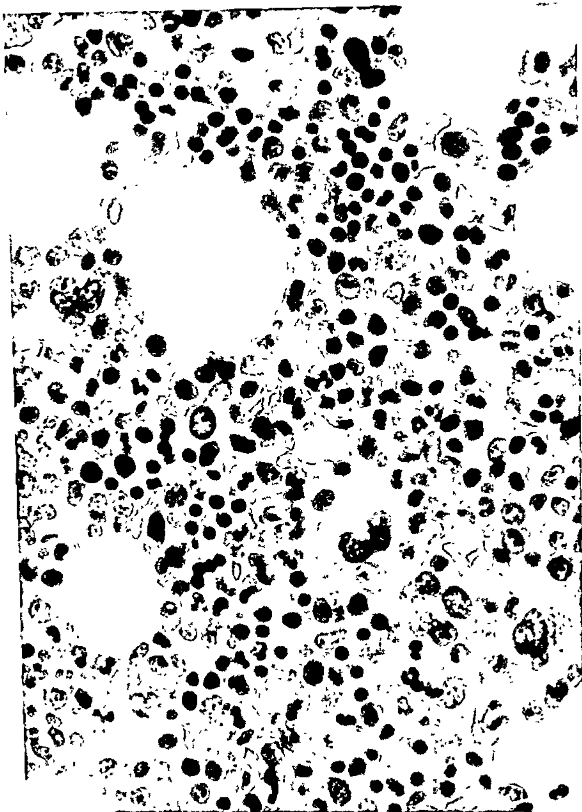


Fig 180 *Infectious Mononucleosis Bone Marrow Section* The marrow displays moderate hyperplasia of all three developmental series The small cells with dark nuclei are red cell progenitors, *not lymphocytes*, and are somewhat more numerous here than one finds in the average case where cells of the granulocytic series predominate A cluster of megakaryocytes is seen in the lower right Lymphocytes are not present in the tissue, but are found in marrow smears due to admixture of peripheral blood (× 1000)

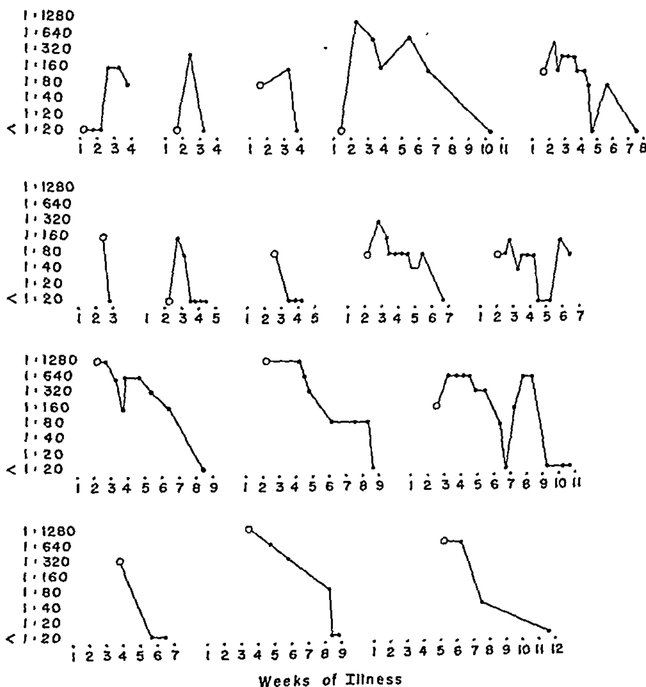


Fig 181 (From the data of W H Walker, by courtesy of the editors of the Bulletin of the U S Army Medical Department)

Leukopenia is often directly proportional to the severity of the disease, and I have seen counts below 1000 per cu. mm. in fatal cases. Cells of the granulocytic series are primarily reduced, band cells are relatively numerous, and degenerative changes in neutrophils may be pronounced. Neutrophilic leukocytosis generally indicates a superimposed pyogenic infection, although intestinal involvement is said to provoke a similar reaction. In uncomplicated cases, neutrophil levels are slow in reaching normal during convalescence, and a relative lymphocytosis may exist for some time after apparent recovery. Nonthrombocytopenic purpura has been described in influenza.

The blood picture in *dengue* (breakbone fever) is a fairly constant one. Beginning on the second day of the disease, both neutrophils and mononuclear cells show a progressive decrease, reaching a low point about the fifth day with a total count between 2000 and 3000 per cu mm A large proportion of neutrophils display immaturity and degenerative changes, the left shift having begun a day or so before the onset of fever. After the symptoms have subsided, it requires a few days for normal leukocyte levels to be restored. In *pappataci fever* (Mediterranean dengue, sandfly fever) lymphocytes decrease sharply during the first day of fever, then begin a slow rise which reaches the normal level in the postfebrile period. Segmented neutrophils fall soon after the lymphocytes, and immature neutrophils take their place, outnumbering them for a few days, then slowly disappearing as fever declines and mature neutrophils return to normal.

Hematologic findings in *psittacosis* and *atypical (virus) pneumonia* resemble those of influenza very closely, and are of virtually no assistance in the differential diagnosis.

Infection with *neurotropic viruses* (poliomyelitis, rabies, herpes zoster, and some of the encephalitides) is frequently marked by neutrophilic leukocytosis, at least in the earlier stages of the disease.

Bone Marrow in Virus Diseases. One can generalize that infection with viruses provokes little or no reaction in the bone marrow. Except for severe smallpox with anemia, where erythropoietic hyperplasia may be extensive, the usual finding is a slight to moderate increase of cells of the granulocytic series, sometimes accompanied by megakaryocytic proliferation. The perivascular round cell reaction common to affected tissues elsewhere is not evident in the marrow.

RICKETTSIAL DISEASES

Rickettsias are minute pleomorphic organisms that occupy a position midway between viruses and bacteria. They resemble viruses by requiring the presence of living cells for their growth, and (possibly excepting the rickettsias of Q fever and trench fever) being obligate intracellular parasites. They are visible through the ordinary microscope, however, when stained appropriately (Giemsa or Macchiavello technics). The pathogenic strains cause diseases that have been divided into the following groups: (1) typhus, (2) scrub typhus (tsutsugamushi fever), (3) spotted fever, (4) Q fever, and (5) trench fever, each with its subgroups. The rickettsioses are transmitted by lice, ticks, and mites. The final diagnosis depends on specific serologic reactions, assisted by animal inoculation. The organisms are more readily demonstrable in the tissues of inoculated animals, especially in smear preparations, than in infected humans.

Hematologic features of the rickettsioses are of little significance. Anemia has not been noted unless nutritional deficiency or some other unrelated cause exists With the exception of spotted fever, leukopenia with either a relative lymphocytosis or normal differential count is the rule. Neutrophilic leukocytosis occurs with fair uniformity in spotted fever, and in the other groups complicated by pneumonia or other pyogenic infection.

The *bone marrow* in spotted fever generally shows slight to moderate hyperplasia of granulocytic components. In the other groups, the marrow displays little change in the absence of complications, although I have sometimes noted reticulo-endothelial hyperplasia and occasional focal necrosis in cases of typhus and scrub typhus. Much of the typhus material available for our study, however, came from concentration camps where the patients were either starved or tuberculous, or both, so that

the marrow pictures were more typical of these other conditions than of typhus per se (Fig. 182). I have not had the opportunity of examining marrow smears for rickettsias, but have had no luck in demonstrating them in marrow sections.

SPIROCHETAL DISEASES

Relapsing Fever

The relapsing fevers are caused by various strains of a loosely wound spirochete with tapering ends, transmitted through lice and ticks. They are characterized clinically by recurring periods of fever and a variety of associated symptoms lasting four or five days, ending abruptly only to recur three to ten days later. there may be two to ten or more such episodes. Spirochetes are generally demonstrable in the peripheral blood (Figs 183 and 184), during the period of fever, especially on the second day, but not during the interval.

Neutrophilic leukocytosis to levels of 15,000 or 20,000 per cu. mm. marks the febrile stages, while the apyrexial phase is frequently characterized by leukopenia with a relative lymphocytosis. Protracted cases generally show a moderate grade of anemia, and thrombocytopenia is a still more constant feature.

Rat-Bite Fever. This is another form of relapsing fever caused by *Spirillum minus*, a short, thick, rigid spirochete not readily found in the peripheral blood. It is transmitted by the bite of infected rats. The clinical and hematologic features are similar to those already described.

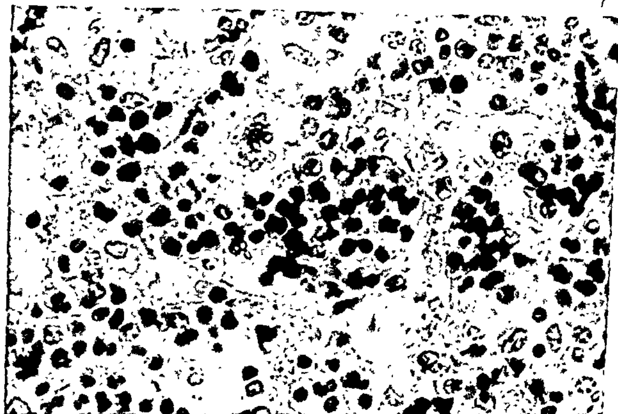


Fig 182 *Epidemic Typhus Bone Marrow Section* Marrow spaces are completely replaced by hematopoietic tissue, mostly red cell progenitors in later developmental stages Reticulo-endothelial elements are also prominent A megakaryocyte is seen in the upper center The erythropoietic hyperplasia is chiefly a reaction to severe hypochromic anemia, resulting from starvation, and not due to typhus per se (× 1000)

Leptospiral Jaundice (Weil's Disease)

Leptospiral jaundice is acquired by ingestion of material contaminated with the excreta of rats infected with *Leptospira icterohaemorrhagiae*. The major clinical manifestations are fever, jaundice, hemorrhage, and an enlarged, tender liver. The organisms are present in the blood stream during the first few days of the

Fig. 183 *Relapsing Fever, Blood* A routine blood smear taken on the second day of a febrile period shows a few delicate spirochetes (*Borrelia carteri*) scattered among the red cells (× 2280)

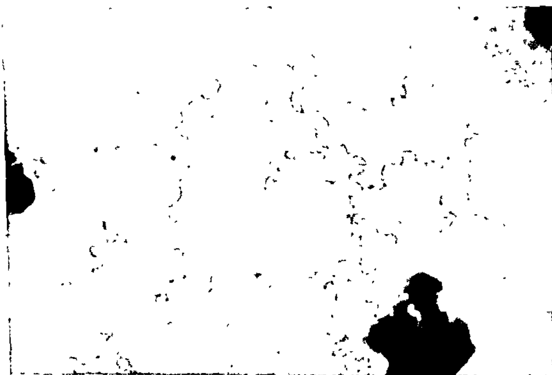


Fig 184 *Relapsing Fever* (same case as Fig 183) *Blood, Thick Drop* Taken at the same time as the blood smear, this thick-drop preparation stained by the Giemsa technic discloses myriads of spirochetes This procedure is especially useful when relatively few organisms are present in the circulating blood (× 2280)

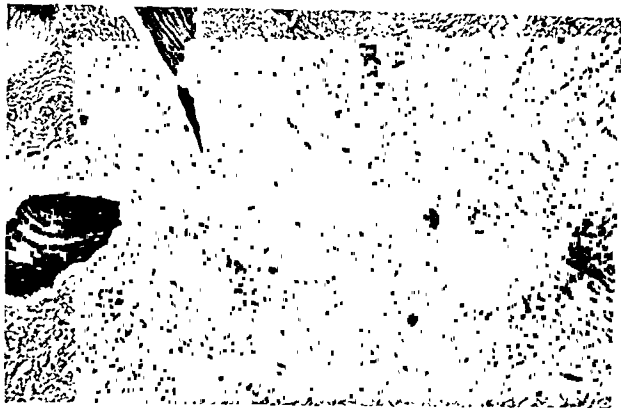


Fig. 185 *Syphilis. Bone Marrow Section.* Several spicules of resorbing bone are seen in the left of the picture The marrow spaces are replaced by loose connective tissue; the more homogeneous portions are areas of gummatous necrosis, a few giant cells being present along the margin The patient had a marked normocytic hypochromic anemia (× 150)

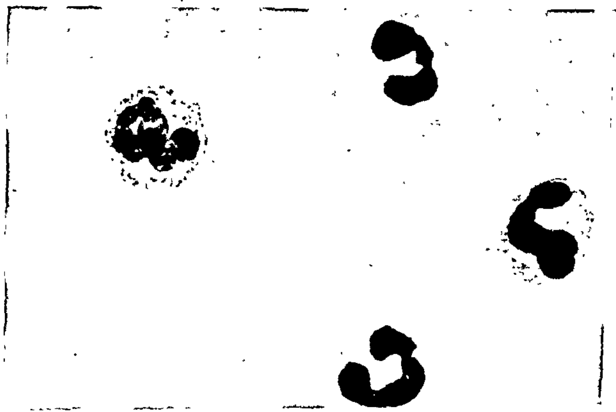


Fig 186. *Septicemia* (Micrococcal) *Blood* Hyperleukocytosis accompanied this blood stream infection with hemolytic *Micrococcus pyogenes* (*Staphylococcus aureus*) The count reached 56,000 per cu mm; neutrophils 91 per cent (myelocytes 4, metamyelocytes 11, band cells 65, segmented cells 11), eosinophils 0, basophils 0, lymphocytes 8, monocytes 1 The neutrophils illustrated are either poorly granulated or contain irregular basophilic (toxic) granules (× 2280)

disease, later in the urine. Leukocytes generally range from 10,000 to 30,000 per cu mm, mostly neutrophils which show a considerable proportion of band cells and metamyelocytes. Anemia is present in the more severe cases, especially when hemorrhage reaches considerable proportions.

Syphilis

The hematologic manifestations of syphilis are as vague and varied as most other features of the disease. It has already been mentioned (Table 22) that congenital syphilis may be responsible for either a neutrophilic or lymphocytic leukemoid reaction. Severe anemia may develop, as well as the syndrome of anemia, leukopenia, and splenomegaly to simulate Banti's syndrome. Paroxysmal hemoglobinuria due to cold has been described (p 171). Anemia is sometimes seen in the secondary stage of the acquired disease, and occasionally in the tertiary. Lymphocytic leukocytosis often occurs in secondary syphilis, while leukopenia with relative lymphocytosis is more common later. Persistent eosinophilia is sometimes seen.

The *bone marrow* appearances are not distinctive except when actual syphilitic osteitis exists (Fig. 185), in which case the bone shows irregular proliferation and resorption, while the marrow is filled with loose connective tissue and often studded with gummas.

Yaws, Betel, and Pinta

No accurate data are at hand regarding blood and bone marrow findings in these diseases.

BACTERIAL DISEASES

Coccal Infections

Infections with cocci virtually always stimulate a neutrophilic *leukocytosis* It is in this class of infection, therefore, that the quantitative and qualitative response of neutrophils best affords an index of bacterial virulence versus host resistance. Thus, a brisk neutrocytosis to moderate heights, with relatively few immature forms and little degenerative change in the cells, suggests a favorable outlook. Leukocytosis, especially to great heights (Fig. 186), with more nonsegmented neutrophils than segmented, and marked "toxic changes" in the neutrophils (Fig. 187), is of more serious import Most ominous of all is early leukocytosis followed by *leukopenia*, where circulating neutrophils show evidence of immaturity and degeneration. Absence of eosinophils and marked reduction in the absolute number of lymphocytes are also unfavorable signs

Recovery is heralded by a decreasing total leukocyte count (or increasing, if it has been low), a shift in neutrophils toward more mature forms of better quality, reappearance of eosinophils (or increase, if they had not disappeared entirely), increase in absolute number of lymphocytes, and a temporary monocytosis.

Marked *anemia* may quickly follow major invasion of the blood stream by hemolytic cocci, probably due both to destruction of circulating red cells and inhibition of erythropoietic tissue of the marrow. The anemia of chronic infection has already been considered (p. 201).

Hemorrhagic phenomena may follow fine embolic deposits in the tissues, as in subacute bacterial endocarditis, or may be due to capillary damage by bacteria or their products. The most spectacular example of the latter is the Waterhouse-Friderichsen syndrome (usually due to fulminant meningococcemia) where there is more or less generalized purpura associated with bilateral adrenal hemorrhage.

It is not feasible to attempt description of the innumerable situations created by coccal infections, varying as they do with the type of organism and its virulence, the location of infection, and the host's response to infection. The *bone marrow* is hyperplastic if infection is severe, cells of the granulocytic series reacting most vigorously.

Bacillary Infections

Infection with *Salmonella typhosa* and other members of the salmonella group generally evokes a slight leukocytosis during the first few days, followed by neutropenia with relative and actual lymphocytosis; sometimes there is an associated monocytosis. *Leukopenia* persists throughout the febrile period; secondary neutrophilic leukocytosis usually indicates a complication, such as hemorrhage or intestinal per-

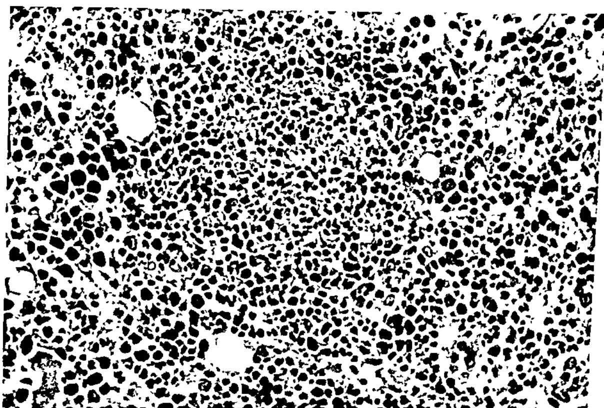


Fig 188. *Typhoid Fever Bone Marrow Section* The patient was suspected of having aleukemic leukemia because of fever, leukopenia, and minor hemorrhagic phenomena, and a bone marrow biopsy was ordered. The marrow was generally hyperplastic and reticulo-endothelial elements were prominent, foci of early necrosis, as seen in the picture, were suggestive of typhoid lesions, and this tentative diagnosis was confirmed by bacteriologic means (× 400).

Fig. 189. *Typhoid Fever. Bone Marrow Section* Another field from the section illustrated in Fig. 188 shows a lesion in which necrosis is more advanced, the center formed by a coarse fibrin mat (× 400).

Fig. 190 *Tuberculosis Bone Marrow Section.* The marrow is extensively replaced by granulomatous tissue with areas [illegible]



Fig 191 *Septicemia* (Clostridium perfringens) *Bone Marrow Section* A young woman developed gas bacillus septicemia after a criminal abortion and died within three days Hemolytic anemia progressed rapidly and the leukocyte count rose to 67,000 per cu. mm , with many metamyelocytes, myelocytes, and occasionally still younger neutrophils appearing in the smears At autopsy, the marrow spaces were virtually emptied of cells of the granulocytic series, as though there had been almost mass delivery into the blood stream Islands of red cell progenitors were conspicuous and showed marked proliferative activity Megakaryocytes (mid-lower left) were markedly degenerated (× 1000).

foration. Slight to moderate *anemia* occurs in nearly all cases, severe in occasional ones, especially in the event of hemorrhage. *Thrombocytopenia* is often noted during the acute phase, but the purpura sometimes seen is due rather to capillary damage The *bone marrow* in typhoid fever is usually hyperplastic, especially on the part of erythropoietic and reticulo-endothelial components Focal necrosis similar to that in lymph nodes, spleen, and liver may be noted (Figs. 188 and 189).

The widely varied blood picture in *tuberculosis* has already been mentioned (p. 187). Alterations in the blood can seldom be correlated with involvement of the *bone marrow* in the disease process, never with any degree of consistency. The marrow shares the miliary tubercles with other tissues during widespread dissemination of the disease through the blood stream. Much larger and more isolated tuberculomas occur as random metastases from an active focus of infection elsewhere (Fig. 190), and frequently enlarge to destroy the surrounding bone.

Tuberculosis has been thought responsible for the development of diffuse myelofibrosis in a small number of cases, sometimes featured by a leukoerythroblastic blood picture and occasionally thrombocythemia.* I have occasionally noted a pronounced plasmacytic reaction in the bone marrow of patients with extensive tuberculosis not affecting this tissue directly; in several instances, plasmacytes were so profuse that myeloma might have been suspected had the specimen been examined objectively.

The hematologic findings in *leprosy* have been described by Kiang and Choa† as follows· "Anemia, mainly normocytic and hypochromic, increased sedimentation rates; infrequent leukocytosis, of moderate degree; marked shift to the left of the white cell count; with high incidence of eosinophilia, lymphocytosis, and monocytosis."

Brucellosis (undulant fever, Malta fever, Bang's abortus fever of cattle) produces a typhoidal type of blood picture in the more severe cases, with neutropenia, relative and absolute lymphocytosis, and frequently a monocytic reaction. There may be little or no change in mild infections or during periods of remission Slight to moderate microcytic anemia has also been observed. The marrow showed no significant change in the few humans that I have examined, but I have seen granulomas in the marrow of experimentally infected guinea pigs.

Pertussis (whooping cough) is characterized by lymphocytic leukocytosis which occasionally reaches leukemoid proportions (Fig. 172) In the early stages, however, leukopenia is the rule, and it is not until the phase of paroxysmal cough that lymphocytosis appears, so that the blood picture is of little aid in diagnosis. Associated neutrophilic leukocytosis generally indicates the development of bronchopneumonia The single case of pertussis in which I studied the marrow picture, there was no specific change evident (Fig 173).

Diphtheria of moderate to severe degree provokes a neutrophilic leukocytosis which may reach high figures, with appearance of immature forms. Profound toxemia may inhibit leukocytosis and at the same time induce anemia, giving evidence of a serious prognosis. The favorable effect of antitoxin administration is rapidly reflected in the blood picture, patients with leukocytosis showing a prompt fall in the count. The *marrow* observed in a few fatal cases showed nonspecific hyperplasia of both erythrocytic and granulocytic series.

Gas gangrene is caused by clostridia which liberate a hemolysin. Severe infections, especially septicemia, produce a rapidly progressive hemolytic anemia and neutrophilic leukocytosis which may simulate leukemia (Figs. 168, 169, and 170). The *marrow* shows hyperplasia of both erythropoietic and granulopoietic cells, and occasionally the latter appear to be wiped out (Fig. 191).

The extreme degree of dehydration seen in *cholera* prevents accurate evaluation of the blood picture. Hemoconcentration per se will result in red cell levels of 7,000,000 or 8,000,000 per cu. mm , leukocyte counts of 15,000 to 20,000, and thrombocytes up to 1,000,000. Restoration of normal blood volume usually reveals that an actual anemia exists Leukocytosis much over 50,000 does not offer a very good

* See Crail and associates Blood, *3* 1426, 1948

† Am J Med Sc, *217* 269, 1949

Fig 192. *Disseminated Lupus Erythematosus. Bone Marrow Smear.* A so-called "L E" cell is ringed with a dotted line. It is a neutrophilic leukocyte which has engulfed a round mass of nuclear material; judging from the number of lobes to the marginal nucleus, it is possible that two segmented neutrophils are involved in the process (× 600) (Slide by courtesy of Dr. James W. Kernohan, Mayo Clinic, Rochester, Minnesota)

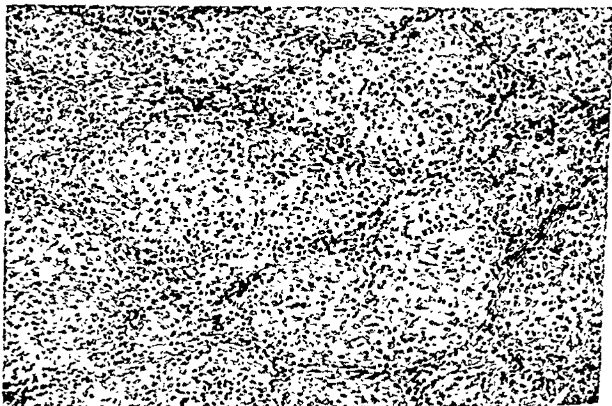


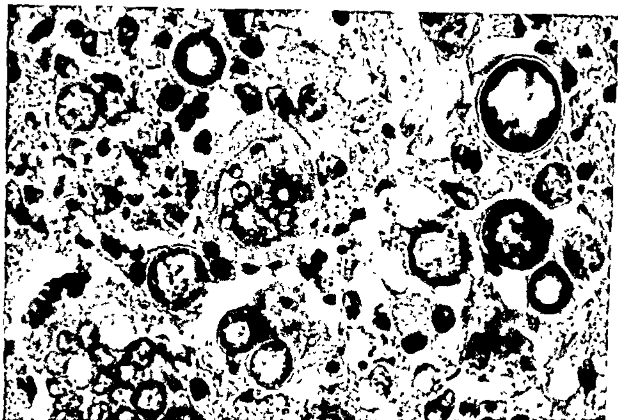
Fig 193 *Boeck's Sarcoid Bone Marrow Section* The patient developed destructive lesions of the phalanges to the extent that multiple fractures rendered several fingers worse than useless, and they were amputated This section shows the marrow cavity of a phalanx completely replaced by granulation tissue characterized by closely packed epithelioid tubercles without caseation necrosis Bone lesions often do not show such clearly defined tubercle formation (× 225).

Fig 194 *Actinomycosis. Bone Marrow Section* A white male of twenty-eight years had severe lower back pain and limitation of motion of the spine, roentgen-ray study showed damage to the bodies of the lower thoracic vertebrae. The leukocyte count early in the disease was elevated to 15,000 per cu mm (mostly neutrophils), but gradually declined to 4100 before death Sections through abscesses found at autopsy disclosed ray fungi of *Actinomyces bovis* (× 700)

outlook, especially when there is a marked shift to the left in neutrophils, and an actual lymphocytosis and monocytosis. The *bone marrow* generally displays nonspecific hyperplasia of the several cell series.

Infection with *Escherichia coli*, *Pasteurella pestis* (plague), and *Pasteurella tularensis* causes neutrophilic leukocytosis.

What has been said of rheumatic fever applies to *chorea*, with the additional observation of eosinophilia in some cases. Eosinophilia is also an *inconstant finding in periarteritis nodosa. Disseminated lupus erythematosus* generally causes anemia and leukopenia, and nonthrombocytopenic purpura is frequently observed. The recently described lupus erythematosus ("L.E.") cell (Fig. 192) is said to be a usual component of the bone marrow in this disease. I have not found it in our cases.



... wide- ... cyst ... to be liberated, elsewhere are spherules in various stages of development. *Budding does not occur (× 1000).*

Ill-Defined Conditions Probably Related to Bacterial Infection

Rheumatic fever is characterized during acute phases by neutrophilic leukocytosis which is roughly proportional to the degree of activity, hypochromic microcytic anemia is frequently associated. Treatment with salicylates in very large doses will produce hypoprothrombinemia, but this seldom results in serious bleeding. One case of thrombocytopenic purpura due to an acquired sensitivity to salicylates has been reported (see Fig. 141).*

Boeck's sarcoid† does not produce any particular change in the blood picture apart from occasional monocytosis or eosinophilia. A good many patients have increased plasma proteins with inversion of the albumin-globulin ratio. *Bone marrow involvement is usually* limited to small bones of the hands and feet and is followed by rarefaction of the affected bones (Fig. 193). Typical epithelioid tubercles

* Rappoport and associates. J. Lab. & Clin. Med., 9:30 16, 1945.

† Etiologic agent unknown, considered here because of superficial resemblance to tuberculosis.

without caseous necrosis may be found, but the granuloma in bone is frequently more diffuse than in other tissues, and the microscopic appearances less typical of the condition.

MYCOTIC DISEASES

Infection of the skin or viscera by pathogenic fungi offers little room for discussion of hem-philic reaction Longstanding infections of systemic type frequently cause hypochromic anemia.

Consideration of these infections is limited to the illustrations (Figs. 194, 195, 196, 197, 198) which show organisms more likely to be encountered in examination of the bone marrow in the systemic mycoses.

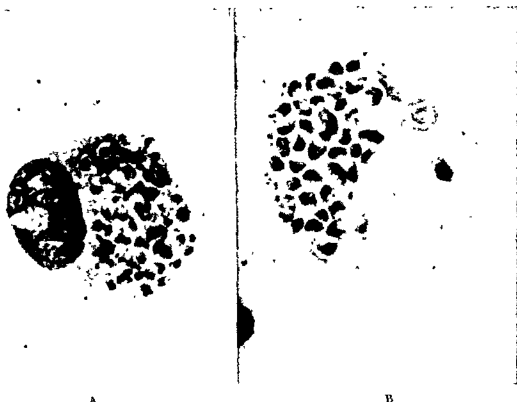


Fig 197 (*Histoplasmosis, Bone Marrow Smear.*) *A*, A macrophage with eccentrically placed nucleus has organisms (*Histoplasma capsulatum*) closely packed throughout its cytoplasm Pale halos representing the capsules are evident around some of the organisms, but the inner structure is not well seen. *B*, Another macrophage which has lost its

Ehrlich, Lebanon Hospital, New York City) (× 2280)

atologic features. One may generalize that the initial infection, if sufficiently extensive, may induce a neutrophilic leukocytosis, and that later stages of the disease may be characterized by actual lymphocytosis or monocytosis. Neutrocytosis is sometimes sustained when the fungus is essentially a pyogenic one, as actinomyces or blastomyces, and involvement of tissues is considerable Sensitization to fungi or their products sometimes evokes an eosino-

PROTOZOAL DISEASES

Protozoa are unicellular animals, a few of which are pathogenic for man. Infection takes place by (1) direct contact with an infected person or animal (trichomoniasis, toxoplasmosis), (2) ingestion of contaminated food or water (amebiasis and other intestinal infections), and (3) biologic transmission by insect vectors (malaria, leishmaniasis, trypanosomiasis). Some infections remain localized (amebia-

sis, giardiasis, balantidiasis, trichomoniasis) or may metastasize (amebiasis); others may be local or systemic intracellular parasites (leishmaniasis, toxoplasmosis). Certain protozoa are primarily blood-borne, either extracellularly (trypanosomes) or within erythrocytes (plasmodia).

Intestinal Protozoal Infections. Infection of the intestinal tract by flagellates, ciliates, and most amebas is seldom reflected in the blood or blood-forming organs. Amebic dysentery

Diagnosis of protozoal infections of the intestine depends entirely on demonstration of the offending organisms in the stools or from swab specimens. Cultures are sometimes helpful.

Kala-Azar (Visceral Leishmaniasis). Kala-azar is a chronic febrile disease produced by a protozoon, *Leishmania donovani*, transmitted mainly by various species of sandfly (Phlebotomus). Cells of the reticulo-endothelial system are extensively involved by the parasite,

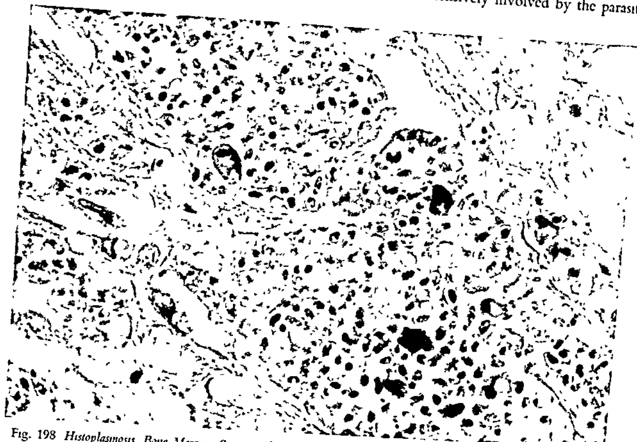


Fig. 198 *Histoplasmosis. Bone Marrow Section* The ... atous lesion of bone in a ... engulfed by macrophages ... 1000)

caused by *Endamoeba histolytica* may produce hypochromic anemia if ulceration is sufficiently extensive to result in significant chronic blood loss Minor neutrophilic leukocytosis is fairly usual in the acute phase and during the invasive phase of amebic hepatitis; monocytes may also be increased in the blood. Multiple small liver abscesses also evoke neutrocytosis, while large solitary abscesses seldom do. Eosinophilia is uncommon, rarely over 10 per cent

and undergo marked hyperplasia, leading to enlargement of the spleen, liver, and usually lymph nodes The bone marrow is likewise affected. The peripheral *blood* shows anemia, leukopenia, and thrombocytopenia, which become progressively worse as the disease progresses, and follow the degree of splenomegaly rather closely. The leukopenia appears first, and is due to a reduction in neutrophils, sometimes proceeding to complete agranulocytosis.

The *bone marrow* is hyperplastic apart from the reticulo-endothelial reaction, with the erythrocytic series particularly abundant. Granulocyte progenitors are present in usual numbers, but there is a poverty of segmented neutrophils and eosinophils. Megakaryocytes appear in normal proportions or less; thrombocyte production is distinctly diminished in much the same fashion as one finds in thrombocytopenic purpura. Effective treatment is followed by restoration of normal structure and function of the marrow, frequently with temporary overproduction of granulocytes and thrombocytes.

Occasionally the organisms are resistive to the usual treatment with antimony compounds, and a state of hypersplenism develops as a result of unchecked hyperplasia of the reticulo-endothelial elements; under these circumstances removal of the spleen is required, and is usually followed by striking improvement.

The *diagnosis* depends on identification of the organisms, which can frequently be found in monocytes and macrophages in blood smears, and almost invariably in spleen or marrow aspirates, as leishmanial forms (Figs. 199, 200, 201). Blood cultures on N.N N. medium will frequently disclose leishmania when they are not discovered on direct observation of smears, in culture the organisms adopt the leptomonad (flagellate) form. The aldehyde and antimony tests, based on increase of euglobulin fraction in blood plasma, are sometimes helpful.

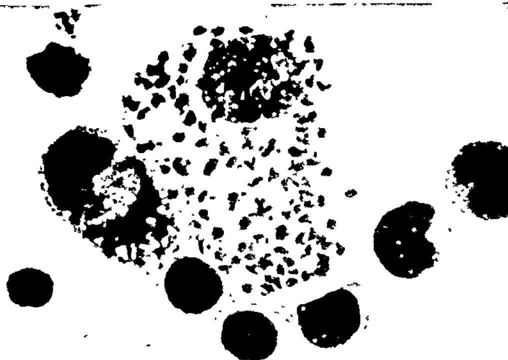


Fig 199. *Kala-Azar* (Visceral Leishmaniasis) *Bone Marrow Smear* A huge macrophage heavily laden with *Leishmania donovani*, the structural details of which are not clearly seen. The patient was a soldier who had acquired the infection in Sicily, his spleen was moderately enlarged, and examination of the blood disclosed anemia and neutropenia with relative and actual monocytosis The diagnosis was made by the finding of organisms in the sternal aspirate, repeated study of the blood having failed to show them (× 2280)

Toxoplasmosis. Hematologic manifestations of infection with *Toxoplasma gondii* are insignificant, and the disease is considered here because of the superficial resemblance of the organisms to aflagellate tissue forms of leish-



Fig 200. *Kala-Azar* (Visceral Leishmaniasis). *Bone Marrow Smear.* Liberated organisms from a disintegrating macrophage show distinct nuclei and cytoplasm, most containing small, peripherally placed kinetoplasts (× 2280).

Fig 201 *Kala-Azar* (Visceral Leishmaniasis) *Bone Marrow Section* Cells of the reticulo-endothelial system are markedly hyperplastic, and the majority contain leishmania (× 1750).

mania and trypanosoma, from which they must be differentiated (Fig 202). In infants, the infection has apparently been transmitted in utero from usually asymptomatic mothers, and produces a granulomatous meningo-encephalitis followed by hydrocephalus. The adult form is a generalized exanthematous process with interstitial pneumonitis and involvement of heart muscle. Organisms are said to have been observed within macrophages in the peripheral blood on a few occasions.



Fig 202 *Toxoplasmosis.* Smear from the brain of an experimentally infected mouse A cluster of eleven organisms occupy the cytoplasm of a macrophage Stained by the May-Grunwald-Giemsa technic, they are seen to be spindled or crescentic, with pale blue cytoplasm and a purplish-red cigar-band nucleus; a light orange zone borders one edge of the nucleus (× 2100)

Trypanosomiasis African trypanosomiasis is caused principally by *Trypanosoma gambiense*, with the other species *Trypanosoma rhodesiense* and *Trypanosoma brucei* being prevalent in certain districts Various species of tsetse fly (Glossina) are vectors from human and animal reservoirs Fever, rash, and lymph node enlargement (especially posterior cervical chains) mark the earlier phases of the disease, while symptoms referable to the central nervous system characterize the later stage. The leukocyte count is seldom increased, but a relative and actual monocytosis is frequently noted Trypanosomes may be found in the peripheral blood (Fig. 203), spinal fluid, or lymph node aspirate; they are difficult to demonstrate in tissue sections. The best means of finding the organisms is by centrifugation of citrated blood and examination of the wet sediment, where motility of the trypanosomes disclose their presence.

South American Trypanosomiasis (Chagas' disease) is an infection with *Trypanosoma cruzi*, [illegible]

[illegible] while only the trypanosomes appear in the peripheral blood. *Trypanosoma cruzi* differs from the other trypanosomes by its more rigid C or U shape, its large kinetoplast and narrow undulating membrane, and absence of dividing forms in the peripheral blood.

The principal damage is to the heart, the muscle of which is heavily infested with leishmanial forms; other organs show chiefly the effects of heart failure.

Malaria. Malaria is the most prevalent infectious disease in the world, although it is relatively isolated to certain regions by reason of low human reservoirs and public health control of vectors elsewhere. Monographs on malariology make anything but a brief mention of the disease unnecessary in this book.

malariae every seventy-two hours; while falciparum is more irregular, sometimes averaging forty-eight hours, sometimes producing continuous or remittent fever. Frequently infections are superimposed, so that parasites mature at different times and upset the schedule.

The thick-drop technic for finding plasmodia in the blood is far more fruitful than examination of the average thin blood film, especially in light infections. Thin films are



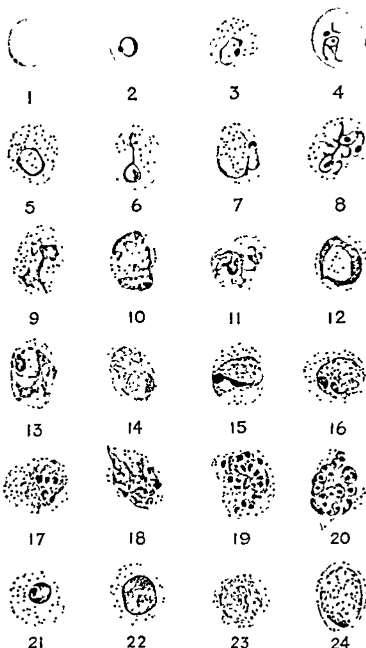
Fig 203 *Trypanosomiasis* (African Sleeping Sickness) *Blood* The trypanosomes (*Trypanosoma gambiense*) are seen as slender wavy flagellates with pointed anterior and blunt posterior tip The large nucleus is centrally placed, and the kinetoplast lies posteriorly to form the origin of an axial filament which borders the undulating membrane and terminates anteriorly as a flagellum (× 2100)

The several species of Plasmodium which affect man (vivax, malariae, falciparum, and rarely ovale) undergo a sexual phase of development in a definitive host, the female anopheline mosquito, while man is the intermediate host for the asexual cycle

The classical cyclic intermittent fever, preceded by chills and followed by sweats, occurs only when all parasites in the blood mature simultaneously, thus, a pure, single vivax infection causes fever every forty-eight hours,

frequently required, however, to demonstrate finer details required in accurate identification of the type of parasite (Figs 204, 205, 206). The tabulation on page 234 from Mackie, Hunter, and Worth* is useful in classifying plasmodia seen in thin stained blood films The many possible differences in severity and outlook require precise diagnosis of the type of infection

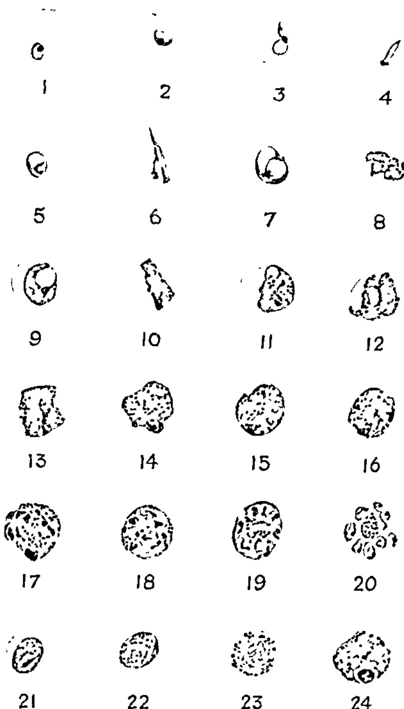
* Mackie, T T, Hunter, G W, and Worth, C. B A Manual of Tropical Medicine, 1945, p 246



INEZ DEMONET

Fig 204 *Plasmodium vivax* (Courtesy National Institute of Health, U.S P H S.). 1 Normal sized red cell with [illegible]

6, 7 Very tenuous medium trophozoite forms 8 Three ameboid trophozoites with fused cytoplasm 9, 11, 12, 13 Older ameboid trophozoites in process of development 10 Two ameboid trophozoites in one cell 14 Mature trophozoite 15 Mature trophozoite with chromatin apparently in process of division 16, 17, 18, 19 Schizonts showing progressive steps in division ("presegmenting schizonts") 20 Mature schizont 21, 22. Developing gametocytes 23 Mature microgametocyte 24 Mature macrogametocyte



INEZ DEMONET

Fig 205 *Plasmodium malariae* (Courtesy National Institute of Health, U S P H S) 1. Young ring form trophozoite of quartan malaria 2, 3, 4 Young trophozoite forms of the parasite showing gradual increase of chromatin and cytoplasm 5 Developing ring form trophozoite showing pigment granule 6 Early band form trophozoite—elongated chromatin, some pigment apparent 7, 8, 9, 10, 11, 12 Some forms which the developing trophozoite of quartan may take. 13, 14 Mature trophozoites—one a band form 15, 16, 17, 18, 19 Phases in the development of the schizont ("presegmenting schizonts") 20 Mature schizont. 21 Immature microgametocyte 22 Immature macrogametocyte 23. Mature microgametocyte 24 Mature macrogametocyte.

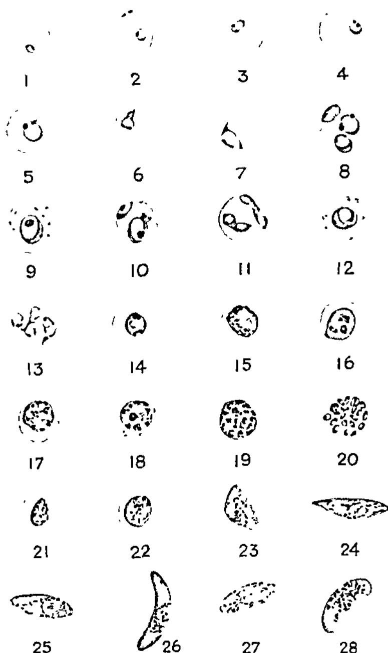


Fig 206 *Plasmodium falciparum* (Courtesy National Institute of Health, U S P H S.) 1 Very young ring form tropho-

Fig 207. *Malaria (Plasmodium knowlesi* in rhesus monkey). *Blood.* Malarial pigment and several red cell fragments are seen in the cytoplasm of a monocyte As pigment-bearing monocytes appear more commonly in textbooks than in blood smears from human malaria, this animal preparation was selected for demonstration (× 2280).

Fig 208 *Malaria (Plasmodium knowlesi* in rhesus monkey) *Blood* The largest cell is a monocyte with extended pseudopods, the vacuolated cytoplasm contains malarial pigment Of interest is the lymphocyte to the left which, despite its reputed incapability for phagocytosis, has engulfed a number of pigment granules (× 2280)

hematoxylin-eosin stain technic (× 1000)

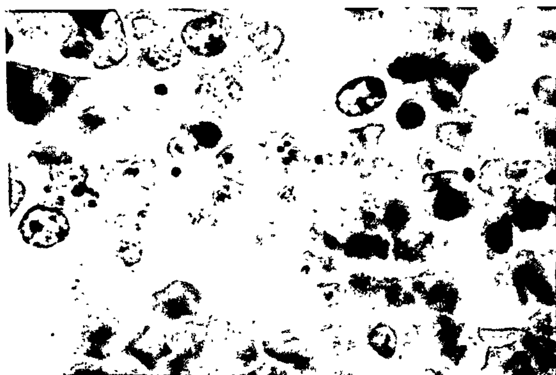


Fig 210 *Malaria* (Plasmodium falciparum) *Bone Marrow Section* Higher magnification to show the profusion of parasitized erythrocytes, evidenced by included pigment granules, the parasites proper not being evident with routine staining methods (× 2280)

TABLE 21

DIFFERENTIAL CHARACTERISTICS OF THE *Plasmodium* IN STAINED FILMS

(Mackie et al.: Manual of Tropical Medicine)

Characteristics	*P. falciparum*	*P. vivax*	*P. ovale*	*P. malariae*
Infected erythrocyte enlarged	−	+	±	−
Infected erythrocyte not enlarged	+	−	±	+
Infected erythrocyte oval, crenated margin*	−	−	+	−
Infected erythrocyte decolorized	−	+	+	−
Infected erythrocyte, Schuffner's dots*	−	+	+	−
Infected erythrocyte, Maurer's dots*	+	−	−	−
Multiple infections in erythrocytes*	+	Rare	−	−
Parasite, all forms in peripheral blood	−	+	+	+
Parasite, large coarse rings	−	+	+	+
Parasite, double chromatin dots*	+	Rare	−	−
Parasite, accolé forms*	+	Rare	−	−
Parasite, band forms*	−	−	+	+
Parasite, crescentic gametocytes	+	−	−	−
Number of merozoites	8–24	12–24	8–12	6–12

* Not invariable but suggestive or diagnostic when seen.

Hemolytic *anemia* is not very evident in malariae infections, but may attain moderate degree in vivax and severe in falciparum. The anemia is generally hypochromic and normocytic, occasionally macrocytic when blood destruction has been rapid. Reticulocyte levels are mostly low while the infection is active, and a crisis usually occurs within ten days after institution of specific therapy; the reticulocyte peak may be sustained for some time in chronic cases. *Leukocytosis* frequently accompanies the malarial paroxysm and the count resumes its normal level during the afebrile period. In chronic malaria, leukopenia is characteristic, and monocytes are generally increased in relative and absolute numbers. The finding of black malarial pigment in monocytes is helpful in diagnosis of light infections, but in actual experience it is not seen often enough to be of much use (Figs 207 and 208) Minor eosinophilia has been observed in some patients during convalescence.

The *bone marrow* is hyperplastic in proportion to the degree of anemia, with the erythrocytic series predominating (Figs 209 and 210). Some observers claim success in finding plasmodia in marrow smears in chronic cases where no organisms could be found in the peripheral blood.

HELMINTHIC DISEASES

A great variety of parasitic worms infect man in their own peculiar fashions, each with its particular life cycle in which man may be the direct or intermediary host. They may be divided, according to the general manner of behavior, as intestinal parasites and tissue-invading parasites; certain of the former have cycles of tissue invasion by their larvae, so that the distinction is not too sharp. Only a few helminthic diseases have been selected for consideration, representing both groups, because of their more distinctive hematologic manifestations. Reference must be made to monographs on parasitology and tropical medicine for details of these and others not mentioned.

Hookworm Disease. The hookworms, *Necator americanus* and *Ancylostoma duodenale*, infect the small intestine of man, their larvae having gained entry either through the skin or by direct ingestion. They cause blood loss and a certain amount of local damage to the intestine at the sites of fixation. While the results of infection are dependent in part on the abundance of parasites, nutrition of the host is of much greater significance. This has been demonstrated in a number of surveys, never more strikingly than among our troops during the recent war; in endemic areas, there was hardly

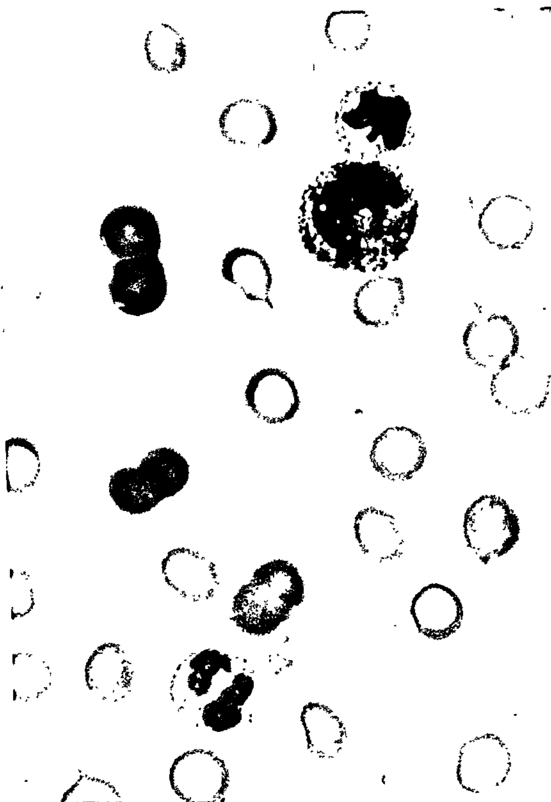


Fig 211 *Hookworm Disease Blood* The patient was a thirteen-year-old underfed boy who was thin, weak, and pallid, and his spleen was markedly enlarged Blood studies revealed severe hypochromic microcytic anemia and eosinophilia, and his stools were full of hookworm ova (*Necator americanus*) The blood smear shows the usual paradox of marked iron deficiency anemia, where most red cells are nearly empty of hemoglobin, while others are richly colored A good diet supplemented with ferrous iron restored blood levels rapidly, splenomegaly regressed, and the patient was subsequently relieved of his worms (× 2280)

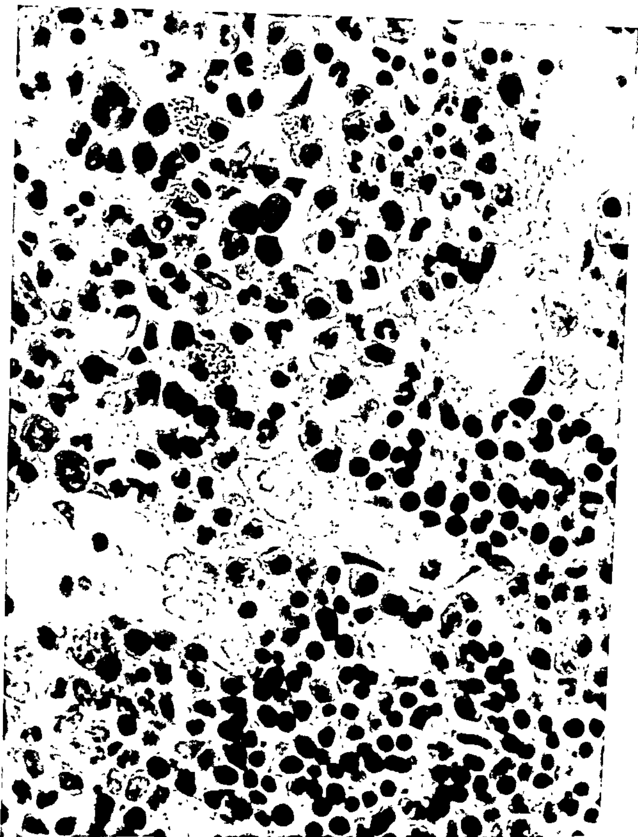


Fig 212 *Hookworm Disease Bone Marrow Section* (same case as Fig 211) The marrow is completely filled with hematopoietic tissue, most cells being intermediate or late forms of red cell progenitors Of the relatively few cells of the granulocytic series, a fairly large proportion are eosinophils, readily distinguished in the print by their coarse granules (1000)

a single case of anemia among infected military personnel, while the more poorly fed natives suffered extensively from the disease.

Stransky and Quintos* observed *three types* or stages of blood changes in hookworm disease. In the *first*, the bone marrow was able to compensate adequately for blood loss, and anemia did not develop; reticulocytosis was present, and the marrow showed erythropoietic hyperplasia. The *second* type was characterized by more or less marked hypochromic anemia (Fig. 211), even though red cell production was accelerated to top degree in the marrow (Fig. 212) (in my experience, marrow activity is frequently bolstered by extramedullary hematopoiesis, especially in the spleen which may enlarge strikingly and regress after effective treatment is instituted). Their *third* type was marked by an aregenerative form of normochromic anemia, leukopenia, and thrombocytopenia, with the marrow relatively poor in hematopoietic tissue. It usually required several years or more for the aplastic type of anemia to develop Stransky and Quintos noted hemorrhagic phenomena in all patients who died, even before marrow aplasia had assumed severe proportions, and regarded the bleeding tendency as a danger signal, it was not observed in patients who recovered Apart from the aplastic group, leukocytosis is usually present, and the proportion of eosinophils may be high.

In patients without anemia, it is only necessary to get rid of the worms by well-standardized means. When hypochromic anemia exists, it is imperative to give a high protein diet and administer ferrous iron before vermifugation; normal blood levels will be restored by these means despite the worms. The aplastic group requires a preliminary series of blood transfusions.

Filariasis. The various species of filarioidea are disseminated by several types of mosquitoes, black flies, tabanid flies, and midges. Threadlike adult worms in the human host give rise to larvae (*microfilariae*) which invade the bloodstream, they are ingested by the blood-sucking insect vector where they undergo further development in preparation for the next victim of the insect's bite.

Infection with *Wuchereria bancrofti* is perhaps the most important one. The adults lodge in the lymphatic system and set up marked inflammatory changes with considerable scar formation. Chronic obstruction to lymph flow results, with consequent enlargement of parts distal to the block, sometimes reaching fantastic proportions (elephantiasis).

Microfilaria of *Wuchereria bancrofti* (Fig. 213) may appear in the peripheral blood in waves during the night (periodic type) or at any time (nonperiodic type). The latter variety seems to be restricted to the South Pacific areas. The *diagnosis* rests with demonstration of microfilaria in the blood or in aspirates from enlarged lymph nodes. Specimens of blood should be obtained between nine and ten o'clock at night to demonstrate the periodic form, while daytime specimens are best for the nonperiodic. Thick-drop preparations stained with hematoxylin and eosin are usually satisfactory, although wet films are frequently useful because movement of the worms calls attention to them; centrifugation of laked blood and examination of the sediment is sometimes required, especially during early or late stages of the disease when the blood is scantily invaded.

Anemia is not a feature of the disease. *Leukocytosis* is inconstantly noted except during inflammatory phases, especially when there is secondary infection with pyogenic cocci. I have seen examples of striking eosinophilia, while other patients have shown none.

Nonpathogenic filaria need be mentioned, as the incidental finding of microfilariae in the peripheral blood is sometimes the cause of needless alarm. Unless the larvae can be identified as those of known pathogens, or there is clinical evidence of disease suggestive of filariasis, their presence can be dismissed with mere notation (Fig. 214). The few such persons that I have observed had lived for some time in Oklahoma or Texas

Trichinosis Infection with *Trichinella spiralis* follows ingestion of raw or partially cooked meat, chiefly pork. In man, the disease is

* Blood, 2 63, 1947



Fig. 213. *Filariasis Blood.* A thick-drop preparation of blood from a native of tropical Australia, stained with hematoxylin and eosin, discloses a microfilaria of *Wuchereria bancrofti*. This is a sheathed worm with smooth head and a tail devoid of nuclei (× 1000)

Fig 215 *Trichinosis Blood.* Thick drop of blood taken during the second week after infection contains a larva of *Trichinella spiralis* (hematoxylin and eosin stain) (× 1000).

characterized by fever, gastro-intestinal disturbances, periorbital edema, muscle pain, and eosinophilia which usually exceeds 20 per cent of a mild leukocytosis and may reach 70 per cent or more of a high one. The actual incidence of the disease in this country approaches 20 per cent of the population, but most infections are so mild as to be unnoticed; significant infections frequently occur in groups of people who have eaten particular batches of heavily encysted and undercooked pork or pork products (hot dogs, bologna, salami, etc.), and mortality is sometimes high.

Ingestion of viable cysts is followed by maturation of the worms in the intestinal tract of the host, fertilization of females, and discharge of larvae which enter the circulating blood and penetrate striated muscle (except for the heart) where they become encysted. This whole process takes place within a few weeks. *Early diagnosis* can sometimes be made by the finding of larvae in the blood, best by the thick-drop technic (Fig. 215); this is generally limited to the second and third week after infection. Larvae have also been demonstrated in spinal fluid. Thereafter, cysts can often be found by biopsy of voluntary muscle (gastrocnemius, deltoid, biceps). Skin and serologic tests are usually reliable after a month, although false positives sometimes occur in *Trichuris trichiura* infections.

Schistosomes and Flukes. The complexity of these groups of parasites prohibits any but brief mention, and only because of their ability as tissue invaders to evoke eosinophilia of considerable proportions.

Cestodes. The one worm in this category that has real hematologic significance is the fish tapeworm, *Diphyllobothrium latum.* Infection is heaviest in countries bordering the Baltic Sea, with a lesser incidence in Japan, French Switzerland, and the Great Lakes region of this country and Canada. In Finland, Totterman* found anemia in the ratio of 1 to every 383 carriers, while von Bonsdorff† noted ninety-six instances of worm anemia among 11,000 medical patients in a military hospital, without knowledge of the incidence of carriers in the group.

The anemia in most cases simulates pernicious anemia, and the bone marrow is megaloblastic; a few have normocytic or microcytic anemia which is sometimes hypochromic, and in these cases the marrow is normoblastic as regards red cell formation. Von Bonsdorff located the site of worms by intubation of a series of patients, and found that those exhibiting the pernicious anemia picture had worms attached at distances of 95 to 135 cm. from the mouth, while those with a non-specific type of anemia or no anemia lay lower in the intestinal tract. The exact manner by which anemia is produced is still not clear.

BARTONELLOSIS

Bartonellae are minute gram-negative organisms apparently unrelated to bacteria, rickettsias or viruses. They are rounded or rod-shaped bodies found in erythrocytes and cells of the reticulo-endothelial system in infected persons. They can be cultivated on appropriate media.

Bartonellosis in Man. The disease (caused by *Bartonella bacilliformis*) is restricted to the northwestern parts of South America well above sea level, in the distribution of the sand-fly vectors. It may adopt a relatively mild cutaneous form (verruga peruana) with the formation of hemangiomatoid skin lesions which may bleed or ulcerate, and usually regress within a few months.

The generalized form of the disease, *Carrión's disease* (Oroya fever), is manifest clinically by fever, malaise, bone and joint pain, and tender enlargement of the liver and spleen. Rapidly progressive macrocytic anemia is generally marked by reticulocytosis, anisocytosis, poikilocytosis, and the appearance of nucleated red cells in the peripheral blood. A large percentage of erythrocytes contain the pleomorphic bartonella bodies (Fig. 216). Neutrophilic leukocytosis is usual. The bone marrow is hyperplastic and displays megaloblastosis, not approaching the degree seen in pernicious anemia, however; reticulo-endothelial components of the marrow are loaded

* Acta med. scandinav., *118* 422, 1944

† Blood, *3* 91, 1948

[illegible]

(× 2280).

Fig 217 *Anaplasmosis Blood* (Cow) The red cells are infested by the organism *Anaplasma marginale* Another form of infection is characterized by centrally placed organisms (*Anaplasma centrale*) (Slide by courtesy of Dr Charles Davis, Denver, Colorado) (× 2280)

with organisms, in common with cells of this system elsewhere. About a third of the cases are fatal within a few weeks of onset.

Bartonellosis in Animals. Infections with bartonellae have been observed in animals, notably *Bartonella muris* in the rat, *Bartonella canis* in the dog, and *Bartonella tyzzeri* in the guinea-pig. Mice are affected by a similar organism, *Eperythrozoon coccoides*, which behaves much like the rat infection in that anemia does not occur, nor do organisms appear in the blood unless the spleen is removed (implying that the phagocytic factor of safety has been lifted). Parasitism of red cells of cattle by *Anaplasma marginale* (Fig. 217) presents somewhat the same picture, although the organism has been classed as a sporozoon; it is transmitted by ticks.

XV

THE LEUKEMIAS

Leukemia is a systemic disease characterized by an unrestricted and purposeless proliferation of leukocytes or closely related cells It is in every sense a malignant neoplasm, and thus far has proved to be invariably fatal. Like other malignant neoplasms, the leukemic cells may adopt any degree of maturity from the most primitive to relative normalcy.

Classification. Leukemia generally follows the pattern set by most malignant tumors in that the more anaplastic the cell type is, the more rapid the course. Thus, leukemia may be classified as *acute* (with predominantly immature cells), *subacute* (with partially differentiated cells), and *chronic* (with mostly adult cells) A course of six months or less is arbitrarily regarded as acute, whereas chronic leukemia implies survival beyond a year, and cases falling into the interval period are listed as subacute One cannot apply these generalizations to each patient, however, for several reasons First, chronic leukemia may exist for some years without the patient being aware of it, and medical attention may be sought only in the terminal phase, so that death occurs within the six-month period allocated to acute leukemia Second, chronic leukemia typified by well-differentiated cells is sometimes punctuated by acute crises during which the predominant cell is of the "blast" type. Under these circumstances, the patient will either die during the crisis or recover to resume the chronic status of the disease. I recall an old lady who survived four such acute episodes and appeared quite healthy the last time I saw her, despite the presence of an enlarged spleen and liver, and a leukocyte count of about 75,000 per cu mm., with most cells segmented neutrophils

Leukemia is also classified as leukemic, subleukemic, and aleukemic, with respect to the blood picture. *Leukemic leukemia* is characterized by pronounced leukocytosis, usually with at least a few atypical cells in the peripheral blood *Subleukemic leukemia* denotes a normal or decreased leukocyte count, some of the circulating cells being abnormal. *Aleukemic leukemia* signifies a normal or moderately deviated leukocyte count, but without immature forms in the blood stream It must be emphasized, however, that alterations in the body tissues are quite the same whether the blood picture be leukemic, subleukemic, or aleukemic.

Finally, leukemia is grouped according to the cell type involved, as follows:

Stem cell	Monocytic
Granulocytic	Plasmacytic
Lymphocytic	Thrombocytic

This classification is not a rigid one For example, stem cell leukemia may in time show a tendency toward differentiation to one or another of the definitive cell series. The cells in granulocytic leukemia may adopt monocytoid characters The thrombocyte series may exhibit striking proliferation during the course of otherwise typical chronic granulocytic leukemia Lymphocytic and moncytic leukemia bear an intimate relationship to lymphosarcoma and reticulum cell sarcoma respectively (Fig 86), granulocytic leukemia to chloroma, and plasmacytic leukemia to multiple myeloma This is all merely a reflection of the fluidity that exists among cells of mesenchymal origin (see Figs 86 and 87).

In an effort to correlate the several classifications of leukemia and introduce a number of the variants, the following tabulation is presented

TABLE 22

CLASSIFICATION OF LEUKEMIA

General Class	*Subclass*	*Predominant Cell*	*Variants*	*Longevity*
Stem Cell	Acute: Leukemic Subleukemic Aleukemic	Undifferentiated		Under 6 mos
Granulocytic	Acute: Leukemic Subleukemic Aleukemic	Myeloblast	Micromyeloblastic Monocytoid (Nageli) Chloroma	Under 6 mos.
	Subacute: Leukemic Subleukemic Aleukemic	Partially differentiated granulocyte		6–12 mos
	Chronic: Leukemic Subleukemic Aleukemic	Well differentiated granulocyte	Subclinical Eosinophilic Basophilic Leukoerythroblastic Polycythemic Thrombocythemic Acute relapsing Myelofibrotic Osteosclerotic	Over 12 mos.
Lymphocytic	Acute Leukemic Subleukemic Aleukemic	Lymphoblast		Under 6 mos
	Subacute: Leukemic Subleukemic Aleukemic	Partially differentiated lymphocyte	Associated with lymphosarcomatous tumor	6–12 mos.
	Chronic Leukemic Subleukemic Aleukemic	Well differentiated lymphocyte		Over 12 mos
Monocytic	Acute. Leukemic Subleukemic Aleukemic	Monoblast		Under 6 mos.
	Subacute Leukemic Subleukemic Aleukemic	Partially differentiated monocyte	Associated with reticulum cell sarcoma	6–12 mos
	Chronic Leukemic Subleukemic Aleukemic	Well differentiated monocyte		Over 12 mos
Plasmacytic	?Acute Leukemic Subleukemic Aleukemic	Plasmablast		
	?Subacute. Leukemic Subleukemic Aleukemic	Partially differentiated plasmacyte	Associated with multiple myeloma of corresponding degree of cell maturity	Unpredictable
	?Chronic Leukemic Subleukemic Aleukemic	Well differentiated plasmacyte		
Thrombocytic	Thrombocythemic Megakaryocythemic			Unpredictable

Incidence. Among autopsy series the incidence of leukemia has ranged from 0.62 to 0.86 per cent, which is probably more nearly correct than the much lower figures that appear in vital statistics Leukemia is said to be on the increase. This is undoubtedly more apparent than real, and is due for the most part to the broader use of clinical laboratory facilities, especially the employment of bone marrow biopsy to identify subleukemic and aleukemic forms of the disease. An actual increase in incidence may well be due to the more extended life span of recent years, whereby a certain number of people survive infection to develop leukemia. Greater use of radiant energy in various forms may also be a factor (see Etiology).

There is a rough correlation between age and the form of leukemia that is apt to develop. Acute leukemia predominates in childhood and young adult life. Chronic leukemia is more [illegible] adults, in common with acute lymphocytic leukemia; acute granulocytic leukemia also occurs with fair frequency before puberty, but is commonly mislabeled, owing to immaturity of the circulating cells. Chronic granulocytic leukemia has its highest incidence between the ages of thirty and sixty years, while chronic lymphocytic leukemia predominates beyond this point. Monocytic leukemia, both acute and chronic forms, is most commonly found in persons over thirty years of age.

The sex incidence is about equal between the ages of ten and forty years. Before and after this period the disease predominates in males.

Etiology In common with most malignant tumors, the cause of leukemia remains obscure. The *hereditary factor* features strongly in leukemia of lower animals, especially mice, but is yet to be evaluated in man. Multiple incidence of the disease, mostly of the lymphocytic variety, has been reported in a small number of families, and some authors have regarded this as more than coincidental. The fact remains that the vast majority of cases have no demonstrable familial linkage.

Less than twenty-five authentic cases of congenital leukemia have been reported, over three-quarters of these were granulocytic in type The condition is probably not as rare as these figures would indicate; some surely go unrecognized, as infants who die at home seldom have blood studies or autopsies performed I have seen two unreported cases, both granulocytic.

Chronic exposure to *radiant energy* seems to play a more definite role as an etiologic agent, as judged by indirect evidence. While leukemia is diagnosed 1 7 times as frequently in physicians as among a comparable lay group of males, the incidence in radiologists is 8 to 10 times that of the nonradiologic group. The apparent increase among physicians at large can be explained on the basis of ready access to diagnostic facilities, but no such excuse can be applied to radiologists. This thesis is supported by the fact that both spontaneous leukemia and susceptibility to transmitted leukemia in mice are increased by irradiation.

A leukemogenic action has been ascribed to protracted but minimal *intoxication* with benzol, aniline dyes, and related compounds, a view which has some experimental support. An individual potential leukemic reactivity is thought by some authors to determine the occurrence of leukemia in the few of many persons subjected to the same occupational hazard. *Endogenous substances* extracted from the urine of patients with granulocytic and lymphocytic leukemia have been separated into carbinol (lymphocytic) and noncarbinol (granulocytic) fractions, each a stimulator of the respective cells when injected into guinea pigs;* it has been postulated that imbalance of these substances may determine the occurrence of leukemia. *Trauma* is regarded by some authors as at least an exciting factor in the development of leukemia. It is more likely that the injury merely served to bring the person under medical scrutiny whereby the leukemia was identified *Virus infection* as a causal agent has not been finally evaluated. Fowl leukosis, which has many features in common with human leukemia, can be transmitted by a cell-free filtrate; no such transmission has been accomplished in man.

Pathology. The lesions of leukemia are more or less widespread, involving primarily the blood-forming organs and extending in a hit-or-miss fashion throughout organs and tissues not concerned with hematopoiesis in postnatal life. The latter foci of cell growth are generally called "leukemic infiltrations" and are regarded by many pathologists as metastases which develop through colonization of circulating leukemic cells. If one recalls that cells of the reticulo-endothelial system retain their hematopoietic potentialities throughout life, and that these cells are distributed among the interstitial tissues of the whole body, it is not difficult to picture them receiving the same stimulus as the blood-forming organs proper to form abnormal leukocytes. I be-

* Miller and Turner Am. J M Sc, 206 146, 1943

lieve that these "infiltrations" are autochthonous and better termed "leukemic metaplasia." This would explain the "infiltrations" that are found with just as much regularity in subleukemic and aleukemic leukemia as in the leukemic form.

The pathologic picture in general is conditioned by the degree and distribution of leukemic cellular proliferation, and hemorrhage. The variation among patients, even those suffering from the same variety of leukemia, is striking. This is illustrated by the splenic weights (all adults) reported by Krumbhaar and Stengel* which ranged from 130 to 920 gm. in acute lymphocytic leukemia, and from 100 to 4400 gm. in the chronic form; spleens in acute granulocytic leukemia varied from 86 to 1650 gm., in chronic granulocytic leukemia from 160 to 4930 gm., and in monocytic leukemia from 160 to 550 gm. The liver is virtually always involved, but does not show such a broad weight scatter as the spleen. The frequency with which other organs and tissues were affected in the series of 123 patients described by Kirshbaum and Preuss† is as follows: kidney 63 per cent, heart 34 per cent, lungs 14 per cent, intestines 13 per cent, adrenals 12 per cent, thymus 10 per cent, pancreas 7 per cent, central nervous system 7 per cent, skin 4 per cent, oral mucosa 4 per cent, thyroid 1 per cent, and urinary bladder 0.8 per cent. In my experience, the incidence of metaplastic foci was considerably greater than this series would indicate, and the distribution broader. Hemorrhage is a constant and usually conspicuous finding in acute leukemia, owing to the almost invariable thrombocytopenia. All organs and tissues may be affected, but hemorrhage is usually most prominent in the skin, along the gastro-intestinal tract, in the central nervous system, and beneath serous surfaces, especially the pericardium. Effusions are commonly noted in the serous cavities and may be serous or serosanguinous. In chronic leukemia, hemorrhage is often minimal or absent, dependent on the preservation of megakaryocytes in the bone marrow.

The *marrow of all bones* [illegible] *emia* (Figs. 219, 220, 222, 224, 246, 247, 248, 251, 262, 263) is the seat of *uniform proliferation* of the immature leukemic cells which more or less rapidly *replace normal hematopoietic tissue* and fat. Early in the disease, normal marrow elements are *richly admixed with the leukemic* ones, but toward the end an aspirate may be made up solely of neoplastic cells. The intramedullary growth is so great that the bony cortex and trabeculae are often thinned.

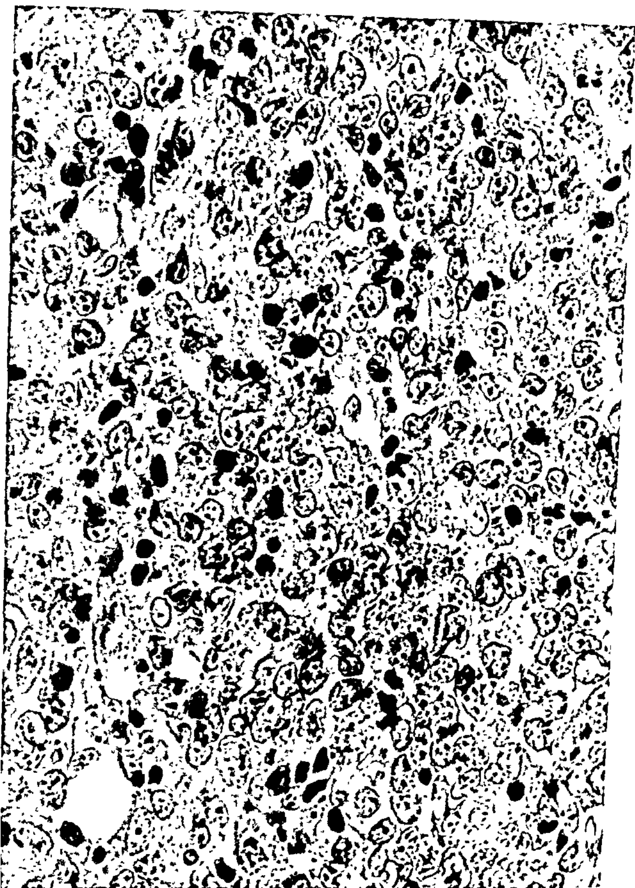
During *remissions* in acute leukemias, either spontaneous or induced by a therapeutic agent such as aminopterin, the leukemic cells of the marrow regress, and occasionally disappear, their place being taken by regenerating hematopoietic tissue of normal character.

A specific variant of acute granulocytic leukemia, *chloroma*, deserves special mention. The leukemic tissue has a grass-green color which fades to pearl gray after exposure to air. In addition to filling the marrow cavities, the green tumor is generally plastered over the outer surface of bones, and may also be found as nodular metastases to the various viscera (Figs. 229, 230, and 231).

In *chronic leukemia*, the bone marrow pattern is much more specific for the affected cell series. *Chronic granulocytic leukemia* will produce a variety of marrow pictures. The most typical one is a myeloid cavity full or nearly full of hematopoietic cells for which the erythrogranulocytic ratio is at most 1:10 and generally well beyond. Instead of an orderly maturation sequence there is an overabundance of cells at each end of the developmental series, i.e. myeloblasts and progranulocytes, and band and segmented forms (Figs. 234 and 239). The mature forms of granulocytes may be so numerous and grouped as to simulate areas of suppuration (Fig. 234). Clumps of nucleated red blood cells are conspicuous until the late stage of the disease when they are more or less overgrown by granulocyte progenitors; mega- [illegible] instances. Dur- [illegible] the [illegible] hat [illegible] the marrow, and with a subsequent [illegible] the more mature state of the tissue is [illegible] an unco [illegible] riant is shown in [illegible] *of the cells are*

* Arch. Path., 34 117, 1942.
† Arch. Int. Med., 71.777, 1943

Fig 219 *Stem Cell Leukemia Bone Marrow Imprint* (same case as Fig 218) The cells are considerably more intact than those observed in blood smears Here nucleoli stand out sharply, and nuclear chromatin presents a fluffy delicacy Cytoplasm is seen as an irregular narrow rim and contains no granules (× 2280)



marrow is completely filled
re is a close similarity to the
e few *residual red cell pro-*
enitors, and sparsely distributed granulocytes remain The same change was observed in all bones examined (× 1000).

Megakaryocyte

Promyelocyte

Stab

Normoblasts

Myeloblasts

Smear (same case as Fig 221) Myeloblasts and
There was a striking paucity of forms intermediate
× 2280)

neutrophilic myelocytes. *Megakaryocytes are* sometimes found *in great profusion* (Fig. 241), occasionally associated *with marked thrombo-*

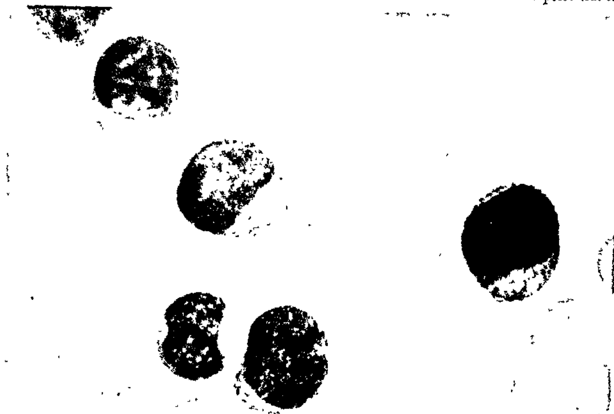
cytic leukemia has already been discussed (p. 84, Figs. 66–69); megakaryocytosis is generally a prominent feature of these conditions. As one would expect, the marrow in chronic eosinophilic leukemia contains a preponderance of this type of cell. When basophils are prominent in the peripheral blood, they are also numerous in marrow smears; sections of the marrow fail to disclose the basophilic granules which are apparently dissolved during the technical procedures

In *chronic lymphocytic leukemia* the bone marrow may be relatively normal for a long time, or may show an inconspicuous interspersion of lymphocytes among the hematopoietic elements (Fig. 254) Often one will find merely a sparse scattering of small lymphocytic aggregates, indistinguishable from the lymphoid *nodules* which are observed incidentally at autopsy in about 10 per cent of older people.

Later, the *foci* of lymphocytic metaplasia assume considerable proportions and ultimately *coalesce* to displace much of the hematopoietic tissue (Fig 256). The intramedullary proliferation may be so marked that bone rarefaction occurs, occasionally with complete dissolution of the cortex and formation of periosteal tumors (Fig. 257). On several occasions I have observed starvation changes (gelatinous degeneration) in the displaced marrow of untreated chronic lymphocytic leukemia (Fig. 259).

Much ado has been made of "lymphosarcoma cell leukemia" (leukosarcoma) as a separate entity.

It is quite true that spillage of tumor cells may account for the blood picture (Fig. 260), but the intimate relationship between the lymphomas that has been demonstrated (p. 94) makes this category superfluous.



leukocytes (× 2280).

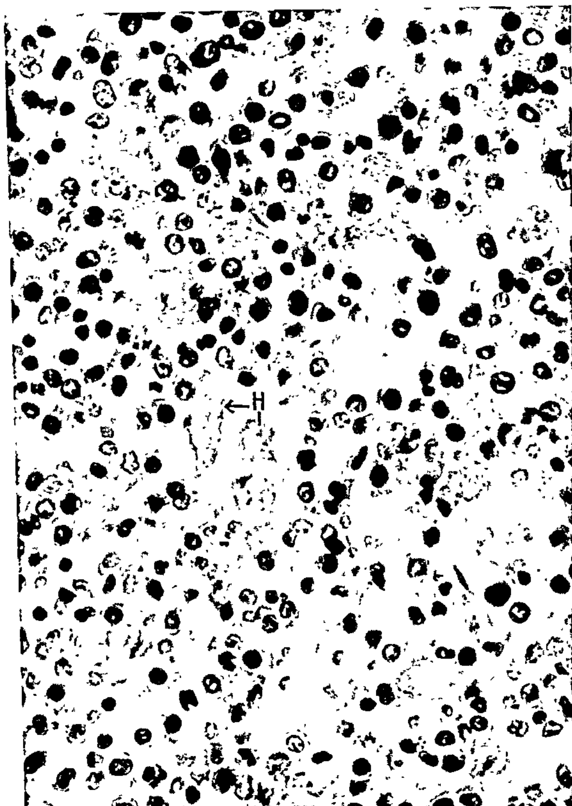
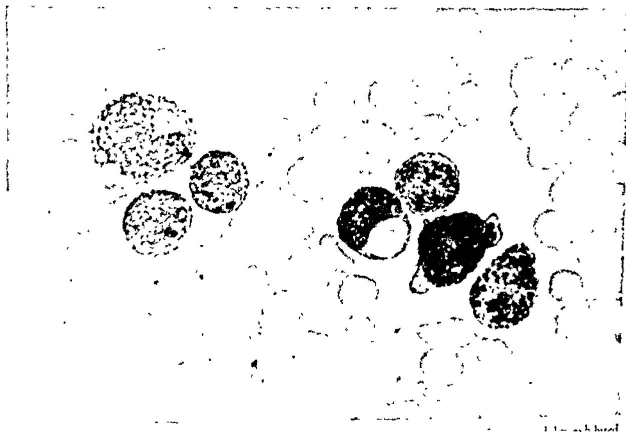


Fig 224 *Granulocytic Leukemia, Acute Bone Marrow Section* (same case as Figs 221, 222, and 223) Most cells are immature granulocytes The smallest cells with black nuclei are normoblasts The marked reticulo-endothelial hyperplasia was stimulated by blood transfusions, and many histiocytes (H) display erythrophagocytosis (× 1000)

I have had no experience with really *chronic monocytic leukemia* and can refer only to cases of rather short duration, but with a fairly well-differentiated type of leukemic cell. Perhaps in these instances the disease had existed long before it became manifest. Such a case is illustrated in Figs. 265, 266, and 267. The circulating cells and those in the bone marrow aspirate are readily recognizable as monocytes, some showing a certain degree of immaturity. The marrow section (Fig. 267) bears a close resemblance to a well differentiated lymphoma of the reticulum cell type, with a scattering of nucleated red blood cells and granulocytes interspersed. These appearances offer a linkage to the so-called "reticulo-endotheliosis" (nonlipoid histiocytosis) as shown in Figs 268 and 269 (Letterer-Siewe's disease when it occurs in infants).

At any rate, this whole group appears to comprise a neoplastic disease in which the cells resemble more closely those of the reticulo-endothelial system than one finds in the other forms of leukemia.

There is some question as to whether *plasmacytic leukemia* should be recognized apart from multiple myeloma. Occasionally the course of the disease may be brief, associated with tumor cells in the peripheral blood, more or less diffuse neoplastic proliferation of plasmacytes in the tissues generally, and no real tumefaction in the bone marrow (Figs. 270 and 271). This probably bears the same relation to



myeloma as lymphocytic leukemia does to lymphosarcoma.

Thrombocytic leukemia is a curiosity. Most cases so labeled have probably been examples of chronic granulocytic leukemia with associated megakaryocyte proliferation (p. 84, Figs. 68, 69, 240, 241). The case pictured in Fig. 272 supports the view that this form of leukemia actually exists. The bone marrow contains a preponderance of megakaryocytes, many of which are bizarre or immature, and

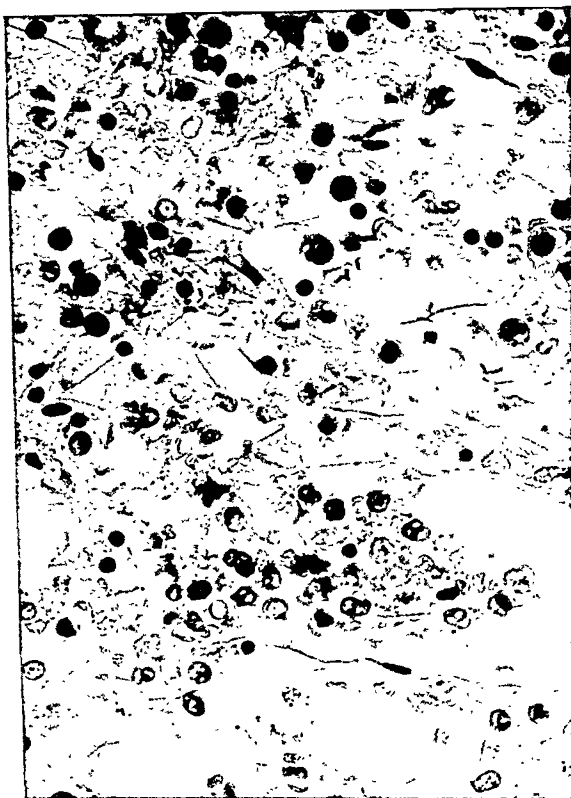
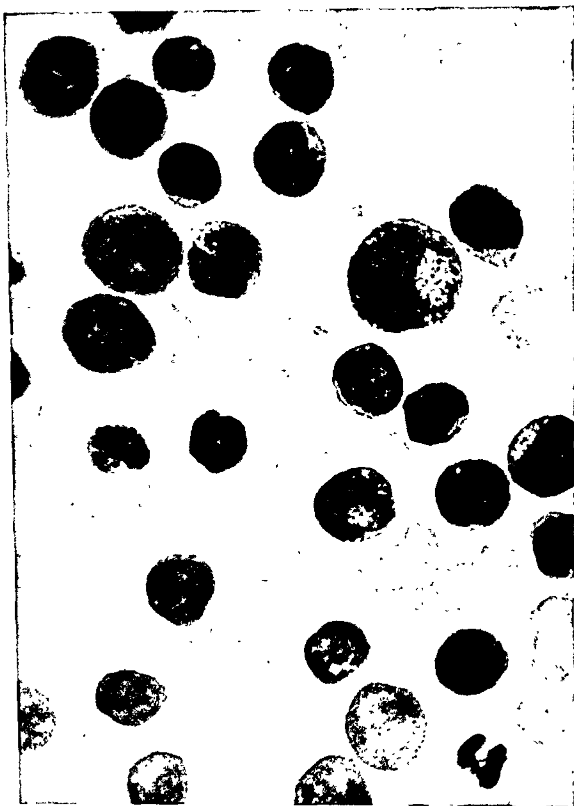


Fig 226 *Granulocytic Leukemia, Acute Bone Marrow Section After Roentgen Therapy* (same case as Fig 225) The myeloid cavity has been largely emptied of immature granulocytes, leaving a sprinkling of the less sensitive nucleated red cells A cluster of degenerating granulocytes remains in a large blood sinus. There is hemorrhage and fibrin deposition in the interstices (× 1000)



Fig 227 *Granulocytic Leukemia, Acute Bone Marrow Section Following Radioactive Phosphorus* (P32) Three millicuries of P32 were given intravenously, and the patient died five days later The total leukocyte count fell from 25,600 per cu mm. (mostly myeloblasts) to 3400 on the day of death. The marrow had previously been solidly cellular, with myeloblasts and progranulocytes predominating, at autopsy it was found relatively empty Fibrin mats surrounded many of the blood channels (lower center), suggestive of endothelial damage, as well (× 1000)



their finely meshed chromatin (× 2280)

there is no concomitant granulocytosis or myelofibrosis.

Symptoms. Leukemia may exist for months or years without any clinical evidence of the disease, and in many instances it has been discovered only when an incidental blood count disclosed an otherwise unexplained hyperleukocytosis. I have seen this happen most frequently among older people with chronic lymphocytic leukemia, and have also found subclinical granulocytic leukemia in a few



Fig 229. *Chloroma. Blood.* The patient was a white female of seventeen years who came under observation because of trifacial neuralgia, followed by facial paralysis of peripheral type. Her leukocyte count rose from 40,000 to 100,000 per cu mm (nearly all myeloblasts) in the course of two weeks, and anemia and thrombocytopenia progressed to very low levels. The field illustrated contains three typical myeloblasts and a large unidentified cell (× 2280).

soldiers in rigorous training and in two young nurses. After varying periods of time the disease became manifest in each patient.

The symptoms of leukemia are exceedingly variable, and no set pattern can be fixed for an individual patient. The symptoms may be divided into two general categories. (1) nonspecific manifestations common to many disease processes, and (2) signs and symptoms directly related to the neoplastic proliferation of leukemic cells. Hemorrhagic phenomena occupy a position midway between the two, and may be red herring in the differential diagnosis of subleukemic and aleukemic leukemias, as will be mentioned later.

The *general symptoms* are frequently the first to be noted. Vague or even sharp pains in the arms or legs often simulate neuritis or arthritis, and abdominal pain has sometimes led to surgical exploration. Complaints referable to the *gastro-intestinal* tract (anorexia, nausea, vomiting, diarrhea) are common. Loss of weight, weakness, tachycardia, sweating, and an elevated basal metabolic rate make up a fairly good picture of toxic goiter, and on several occasions I have seen patients scheduled for thyroidectomy until blood studies settled the matter. Fever may or may not be present during the early phases of the illness, but is almost invariably a feature of the later stages. Ulcerative stomatitis is often regarded as trench mouth and treated as such for some time. Furunculosis is another evidence of lowered



Fig 230 *Chloroma Section Through Anterior Plate of Sternum* (same case as Fig 229) Autopsy disclosed the marrow of all bones examined to be replaced by "green tumor," and the external surface plastered with similar tissue The spleen and lymph nodes were likewise affected and there were nodular metastases to other organs. The upper section of the picture shows the marrow side of the bone, the spaces filled with uniform neoplastic cells (myeloblasts), and the lower portion presents the thick external overlay of the same sort of tissue (× 200)

resistance to infection, especially in the subleukemic forms, and a few of our patients have developed massive carbuncles. A variety of skin lesions apart from true leukemic "infiltrations" are encountered; certain of these are leukemids, some hemorrhagic, and others urticarial. Urticaria is occasionally seen during and after radiotherapy, and is probably due to a sensitivity to the breakdown products of leukemic cells.

Hemorrhage may occur at the site of ulcerative lesions prior to a significant reduction of thrombocytes, but petechiae, ecchymoses, and free bleeding from mucous surfaces generally follow replacement of the bone marrow by leukemic cells, notably in the acute and subacute forms of the disease Thrombocytopenia is the direct consequence of displaced megakaryocytes; the critical level of thrombocytes at which hemorrhage occurs will vary with the patient, but is always well below 100,000 per cu.mm. A number of our patients with acute or subacute leukemia, mostly subleukemic or aleukemic, have been referred from the gynecologic [illegible] becau[illegible] have come from the urologic and gastro-enterologic clinics, as the genito-urinary and gastro-intestinal tracts may be the sites of early hemorrhage. Confusion with idiopathic thrombocytopenic purpura has sometimes led to splenectomy. Recently I was asked to review sections of a spleen removed from a child who had had extensive purpura and severe anemia, but whose leukocyte count was said to have been essentially normal. I was requested to explain why she died several weeks after the operation. The explanation was simple; the spleen exhibited the typical histologic pattern of acute granulocytic leukemia.

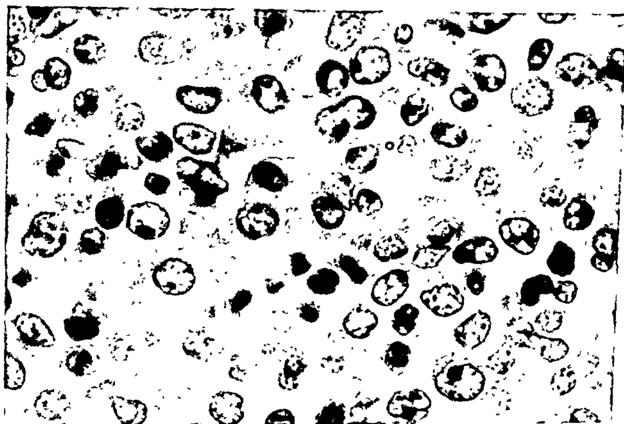


Fig 231 *Chloroma Bone Marrow Section* (higher magnification of Fig 230) Cells vary from undifferentiated reticulum cells (large cell in lower right) to myeloblasts, which predominate. A few nucleated red cells remain (× 1500)

The *growth and spread of leukemic cells* in the bone marrow is also reflected by pallor and weakness, symptoms which appear early in acute leukemia, late in chronic. Pain in bones, often associated with tenderness and sometimes swelling over the bones, is noted more frequently in chronic lymphocytic leukemia, but may occur in any type of the disease.

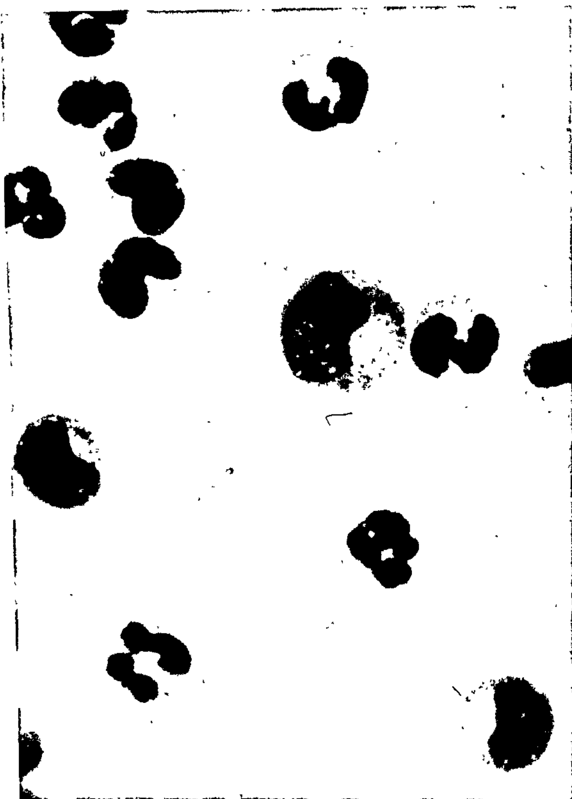


Fig 232 *Granulocytic Leukemia, Chronic Blood* This is a classical blood picture of the disease as it occurred in a fifty-seven-year-old male who "became more readily fatigued than usual" He was slightly anemic and his leukocyte coun [illegible]
few [illegible]
cytes [illegible]

Roentgenograms may or may not disclose diffuse osteoporosis or localized areas of rarefaction. The bone cortex is occasionally destroyed and the invasive growth of leukemic tissue from the marrow can form periosteal tumors (Fig. 257).

The spleen is nearly always enlarged, although in acute leukemia it is frequently not palpable. In chronic leukemia, especially granulocytic, the organ may fill the abdominal cavity. Pain and tenderness over the spleen generally signify infarction. Very large or freshly infarcted spleens may rupture spontaneously or on slight trauma and produce the symptoms characteristic of that catastrophe.

Enlargement of lymph nodes is an inconstant sign. It is more typical of chronic lymphocytic than other forms of leukemia, but even here it may be completely absent. The size of involved lymph nodes often fluctuates, and regression to normal may occur during a remission. During the otherwise conventional course of chronic lymphocytic leukemia large invasive tumor masses may develop, most frequently in the mediastinum or retroperitoneal region, which are indistinguishable in appearance and behavior from lymphosarcoma (see p. 94 and Fig. 86). The same thing can happen in monocytic leukemia where the tumor assumes the appearance of reticulum cell sarcoma. The size and location of enlarged lymph nodes determine the symptoms, if any, which appear. Major enlargement of mediastinal nodes, for example, can produce cough, dyspnea, or hoarseness, and they may impinge on major blood and lymphatic channels to bring about pleural or pericardial effusions and prominent venous patterns on the chest wall. I have seen hydrothorax occur, however, when there has been no demonstrable mediastinal involvement. Obstructive jaundice has followed the pressure of lymph nodes on the bile passages, and severe abdominal pain may be provoked by large mesenteric nodes, especially when the increase in size has been rapid.

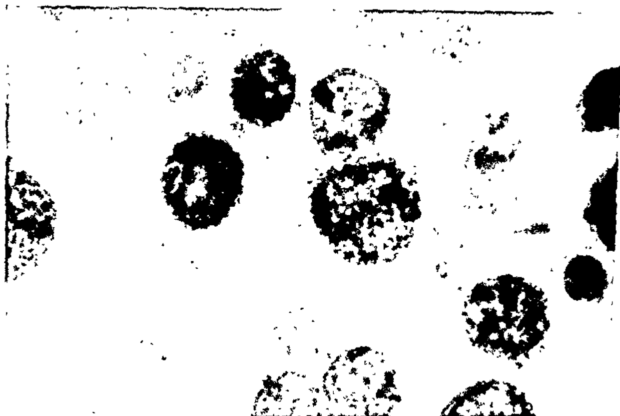


Fig. 233 *Granulocytic Leukemia, Chronic Blood (Peroxidase Stain)* (same case as Fig 232) All cells in the field react positively, the granules being intensely black, except for one at the right border which is a nucleated red cell (× 2280)

The liver is virtually always the seat of leukemic metaplasia and is often enlarged, occasionally massive. In some cases, metaplasia in

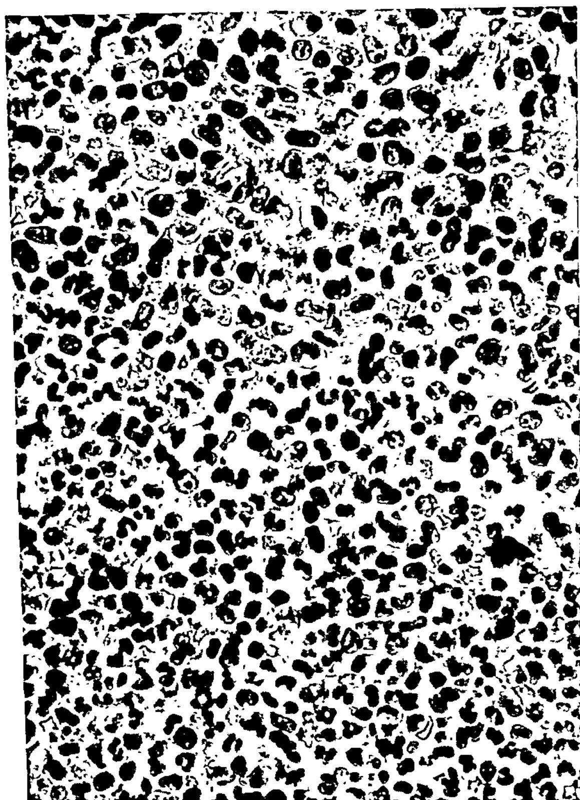


Fig 234 *Granulocytic Leukemia, Chronic. Bone Marrow Section* (same case as Figs 232–234) Section through a large fragment of aspirated marrow fortuitously struck a line of demarcation between a zone of immature granulocytes (upper third) and a large patch of relatively mature ones (lower two-thirds) among which there is a sprinkling of nucleated red cells (× 1000).



Fig 235 *Granulocytic Leukemia, Chronic Bone Marrow Smear* (same case as Figs 232 and 233) The differential count showed granulocytes to predominate 18 : 1, with a disproportionately large number of progranulocytes (promyelocytes) at one end of the scale, and segmented forms at the other (× 2280)

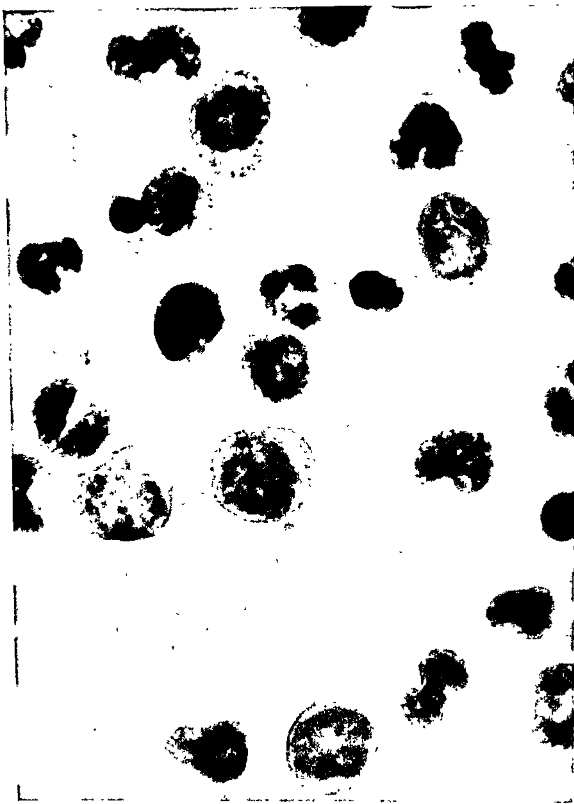




Fig 237 *Granulocytic Leukemia, Chronic Bone Marrow Section* A variant from the usual finding, the marrow in this case shows virtually total displacement of hematopoietic tissue by a uniform mass of neutrophilic myelocytes. Sections stained with hematoxylin and eosin gave the impression of multiple myeloma at first glance, especially as many of the cells have eccentrically placed nuclei, but the Giemsa technic disclosed neutrophilic granules in all cells, which are quite evident even in the black-and-white print (× 1000)

Fig 238. *Granulocytic Leukemia, Chronic, Subclinical Blood* A routine blood count on an apparently healthy young man disclosed a leukocyte count of 35,700 per cu mm with 87 per cent neutrophils, most were adult forms, but there was a generous sprinkling of immature ones, three appearing in the field above. Red cell and thrombocyte levels were normal, the spleen was not enlarged, and the patient felt quite well (× 1350)

Fig 239 *Granulocytic Leukemia Chronic, Subclinical Bone Marrow Smear* (same case as Fig 238) Neutrophils are relatively and actually increased over the normal, and there is a disproportionately large number of progranulocytes These findings tended to support the diagnosis of granulocytic leukemia, and the patient developed clinical evidences of the disease many months later (1350)

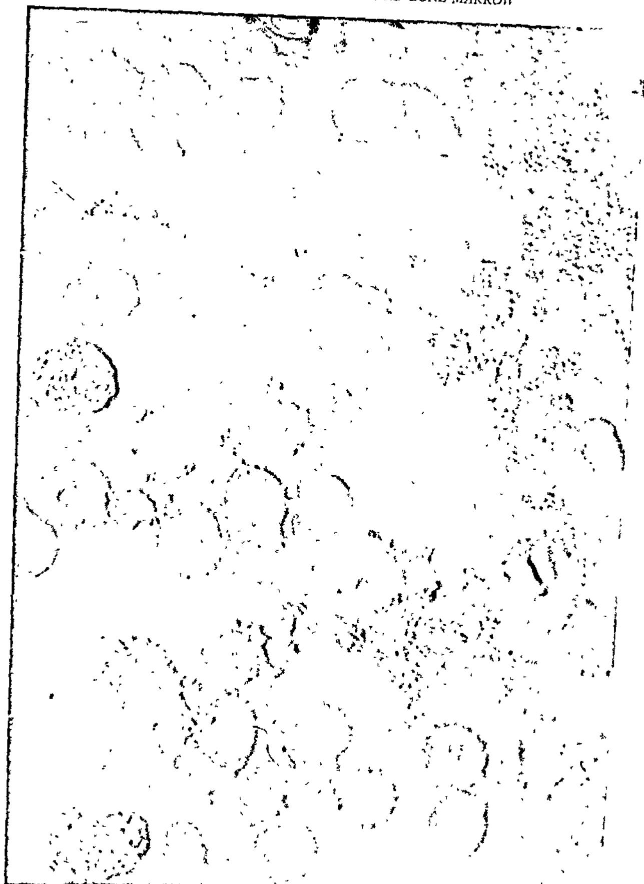


Fig 240 *Granulocytic Leukemia, Chronic, with Thrombocythemia Blood* The patient presented typical clinical and hematologic features of the disease, except that the thrombocyte count consistently remained between 3,000,000 and 3,500,000 per cu mm during the entire period of observation The bas relief photomicrograph shows thrombocyte masses which occupy much of the right third of the field, such masses were richly distributed over the slide (× 2280)

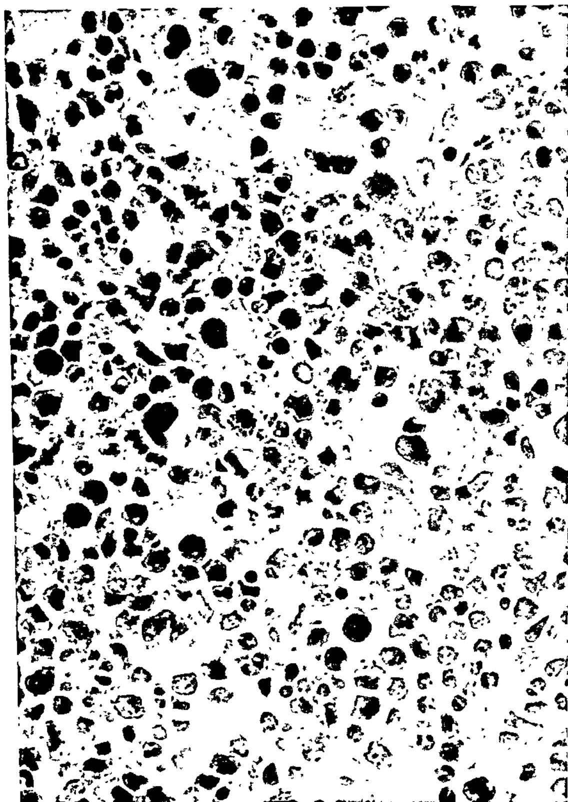


Fig 241 *Granulocytic Leukemia, Chronic, with Thrombocythemia Bone Marrow Section* (same case as Fig. 240) As one

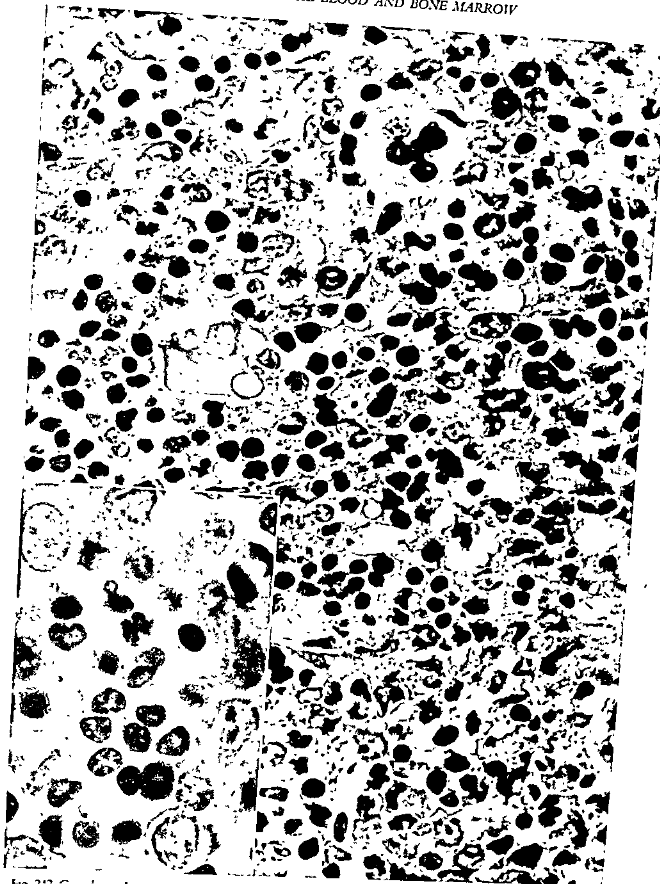


Fig 2[illegible] destroye[illegible] plasia of [illegible] background. The initial leukocyte count was 154,000 per cu.mm and red cells 1,800,000; white cells had fallen to 4900 the week previous to biopsy, and the erythrocyte count was on the upswing (× 1000), insert (× 2000)

the kidneys may be so great as to double their size, and impaired renal function has been noted on occasion. Symptoms referable to the nervous system comprise a variety of palsies, especially in the distribution of cranial nerves, paresthesias, pyramidal tract signs, diminished or absent deep reflexes and manifestations of meningeal irritation. In addition to purpuric spots and the several types of leukemids, nodular lesions of the skin are sometimes seen, I remember one patient who presented the classical appearances of advanced nodular leprosy.

No organ or tissue is immune from either the hemorrhagic or cellular lesions of leukemia, so that the clinical manifestations of the disease are unusually protean The diagnosis in many cases is easily reached, in others it is obscure and rests on careful and repeated examinations of the blood and bone marrow.

Significant Laboratory Data In most cases, the leukocyte count is elevated at some time during the course of the disease, and in rare instances has exceeded 1,000,000 per cu.mm. The very high counts occur in chronic leukemia; those in the acute forms seldom exceed 100,000 per cu.mm and are usually in the neighborhood of 50,000. Some patients, however, present a subleukemic or aleukemic blood picture from beginning to end, whether the leukemia be acute or chronic. The affected leukocyte series nearly always displays immaturity in the circulating blood, varying from a sparse scattering of partially differentiated cells to nearly all blast forms. Exceptions are the strictly aleukemic cases of any type, and chronic lymphocytic leukemia, the latter being characterized by well differentiated lymphocytes, lymphoblasts being rarely seen. Disintegrated (smudge) cells (Fig. 288) are often abundant in the blood films.

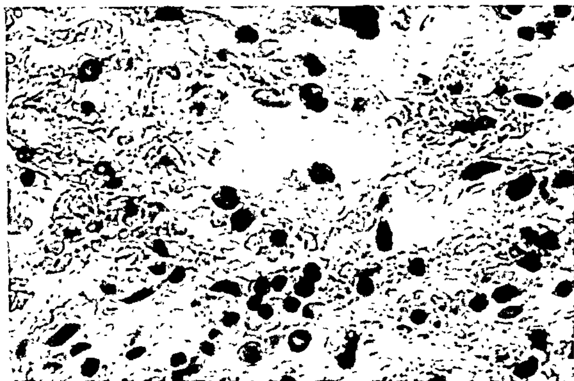


Fig 243 *Granulocytic Leukemia, Chronic. Bone Marrow Section Showing Roentgen Overeffect* The patient had been subjected to fairly heavy roentgen therapy, the exact dosage not known This was followed by a rapid and progressive decline in the blood count, first of granulocytes, then erythrocytes and thrombocytes The marrow is relatively empty of cells, those that remain being red cell progenitors which are less sensitive to radiant energy than granulocytes (× 1000).

The younger the leukemic cell is, the more difficulty one has in identifying its lineage, a situation analogous to humans. In the nursery

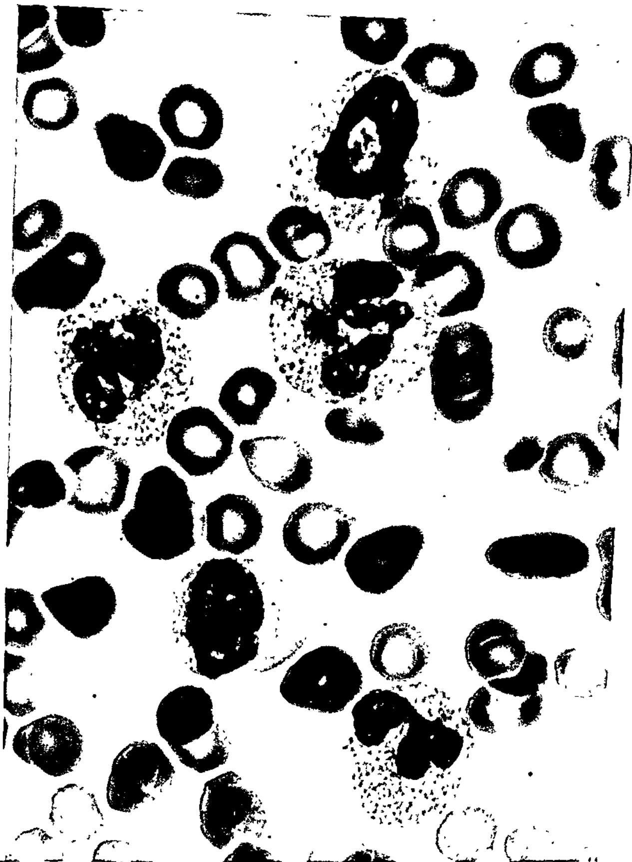
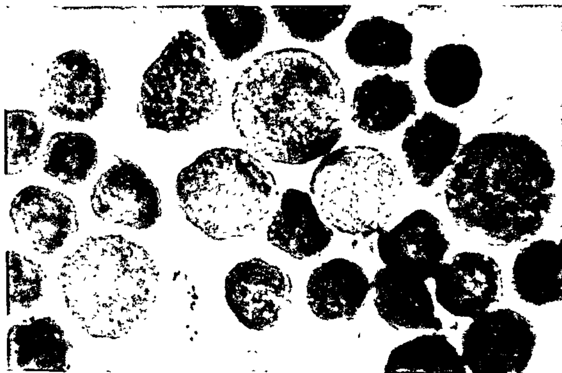


Fig 244 *Granulocytic Leukemia, Chronic, Eosinophilic Blood* The clinical manifestations of the disease did not differ in any respect from those of the neutrophilic type The total leukocyte count ranged between 50,000 and 70,000 per cu mm , with eosinophils comprising 70 to 80 per cent The nuclei of most eosinophils were segmented, although an occasional ring form (top center) was noted. Immature forms (lower left) were seldom encountered A feature of note is the relatively sparse granularity of otherwise adult eosinophils, which may be of assistance in distinguishing leukemic cells from reacting eosinophils (compare with Fig 170) (× 2280).



stage, to settle the matter, we are content to call them "stem cells" (Figs. 218, 219, 220). Given even a slight degree of differentiation, most hematologists have learned to recognize little peculiarities in nuclear and cytoplasmic structure that characterize the definitive cell series, differences that are almost impossible to put into words. Intermediate stages between the primitive and adult forms, when present, are helpful, but in many cases a "leukemic hiatus" exists between the two ends of the developmental line The presence of Auer bodies, pink-

an increase in the absolute number of basophils for several years before the disease becomes manifest. While neutrophils generally predominate in the blood picture, eosinophils (Fig. 244) or basophils are sometimes in the majority. A flood of myeloblasts in the peripheral blood marks an acute exacerbation of clinical symptoms (Fig. 236), but this may subside and all may be well for an unpredictable period. In the [illegible]

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…oblasts, but they may be small (micro-myel-

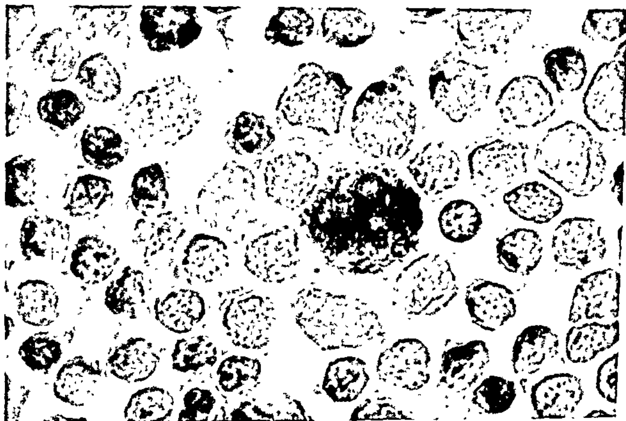


Fig 247 *Lymphocytic Leukemia, Acute, Subleukemic Bone Marrow Smear, Peroxidase Stain* (same case as Figs 245 and 246). All leukemic cells fail to react The peroxidase-positive cell in the center is a residual granulocyte (× 1950).

staining cytoplasmic rods resembling tubercle bacilli, are sometimes found in myeloblasts and monoblasts, and their presence rules out acute lymphocytic leukemia Myeloblasts (Fig. 225) and occasionally monoblasts may engulf red blood cells, thus furnishing an additional clue as to their nature.

Granulocytic leukemia is the notorious faker of the group, frequently existing in its chronic form with a hyperleukocytosis and slight immaturity of granulocytes as the only evidence (Fig 238). Other patients will exhibit merely

oblasts (Fig. 228) and the average technician will call them lymphocytes. Certain cases will be characterized by myeloblasts having mono-

[illegible]

roma cannot be distinguished from the usual one.

The number of thrombocytes invariably diminishes during the course of acute leukemia, often reaching hemorrhagic levels very rapidly. A swing in the thrombocyte count toward

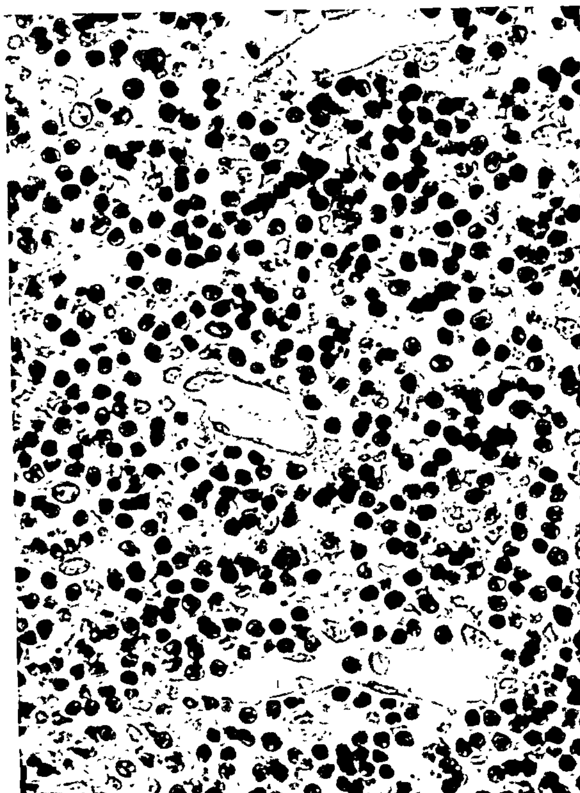


Fig 248 *Lymphocytic Leukemia, Acute, Subleukemic Bone Marrow Section* (same case as Figs 245, 246, and 247) The

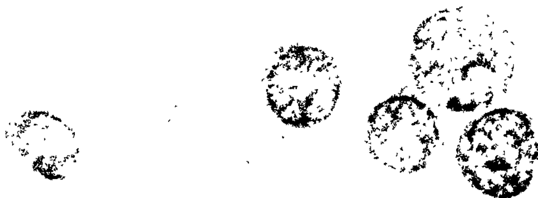


Fig 249 *Lymphocytic Leukemia, Acute, Leukemic Phase. Blood* (same case as Figs 245 to 248). The last few days of life were marked by a flood of leukemic cells into the peripheral blood. A normal lymphocyte lies near the left margin; the other four cells are lymphoblasts (× 2100).

Fig 250 *Lymphocytic Leukemia, Subacute, Leukemic Blood* The patient lived for 16 months after symptoms first appeared The leukocyte count fluctuated between 6000 and 60,000 per cu mm, the low figures following several courses of roentgen therapy (small dosage), large abnormal lymphoid cells constituted nearly all circulating leukocytes A final course of irradiation (doses not known) was followed by a rapid, alarming fall in all formed elements The patient was kept alive for several weeks with blood transfusions, but showed no evidence of regeneration (× 2250)

Fig 251 *Lympho-ytic Leukemia, Subacute, Leukemic Bone Marrow Smear* (same case as Fig 250) Most cells are abnormal lymphocytes, with a few granulocytes and nucleated red cells interspersed (× 2280)

an
he
it
as aplastic anemia (× 1000)

normal is the most reliable sign of remission. Thrombocytopenia is a late manifestation of chronic leukemia, if it occurs at all; hemorrhage is sometimes seen in chronic granulocytic leukemia in the presence of a normal thrombocyte count, probably on the basis of vascular damage and increased permeability. Thrombocythemia, at *times exceeding 3,000,000 per* cu.mm., has been observed in patients with chronic granulocytic leukemia (Fig. 240) as a consequence of associated megakaryocytic proliferation in the bone marrow (Fig. 241). tients who apparently have erythremia and subsequently develop leukemia do not have leukemia from the outset, particularly the ones that terminate in an acute myeloblastic crisis.

The total blood volume is reported greater than normal. The cerebrospinal fluid pressure may be elevated, the protein content increased, *and leukemic cells are often found in the* smears. The urine may contain albumin, casts, and red blood cells, frank hematuria being fairly common in the late stages of acute leukemia. Bence Jones proteinuria has been noted in a few cases. The basal metabolic rate is increased in most cases of chronic granulocytic leukemia and the acute leukemias, as well as during the advanced phase of chronic lymphocytic leukemia; there appears to be a rough correlation between the basal metabolic rate, the total leukocyte count, and the degree of cell immaturity.



Fig 253 *Lymphocytic Leukemia, Chronic, Subclinical Blood* The patient was asymptomatic, and lymphocytosis of 55,000 per cu mm was discovered in a routine blood examination. All lymphocytes were apparently normal mature forms Neither spleen nor lymph nodes were enlarged (× 2100)

Anemia develops with considerable rapidity in cases of acute leukemia. The anemia is generally normochromic and normocytic, but may be slightly macrocytic, and anisocytosis and poikilocytosis are not apt to be very striking. In chronic leukemia, anemia is a latecomer. Indeed, certain patients with chronic granulocytic leukemia may present a markedly increased red blood cell count, and the differential diagnosis between this condition and erythremia can be virtually impossible for a time I wonder sometimes whether those pa-

The uric acid and total nitrogen levels of the blood are elevated and the nitrogen balance may be negative, especially in acute leukemia. There is generally a decrease in total plasma

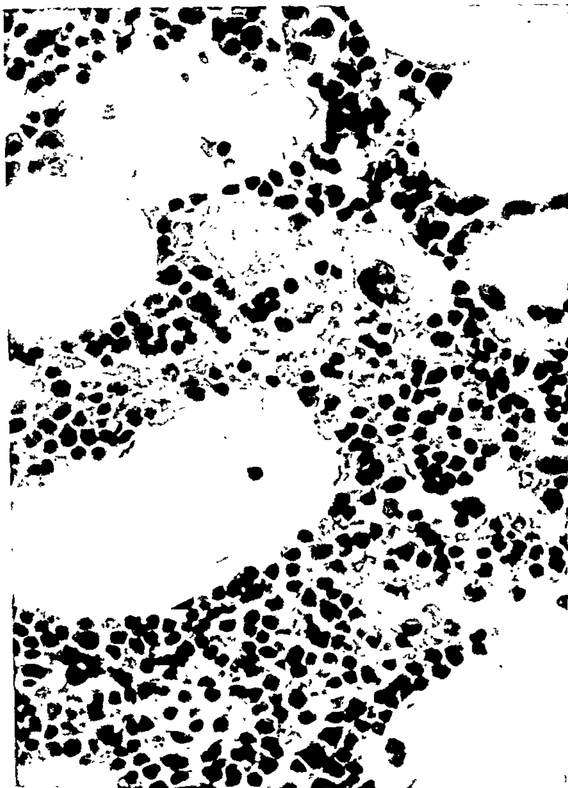


Fig 254 *Lymphocytic Leukemia, Chronic, Subclinical Bone Marrow Section* (same case as Fig 253) At first glance the marrow appeared to show only minor erythropoietic hyperplasia, but closer inspection revealed a scattering of lymphocytes admixed with the normal hematopoietic cells, the findings being insufficient to substantiate the impression of leukemia. Later the spleen and lymph nodes enlarged, and a second biopsy disclosed patchy replacement of the marrow by compact sheets of small lymphocytes (× 1000)

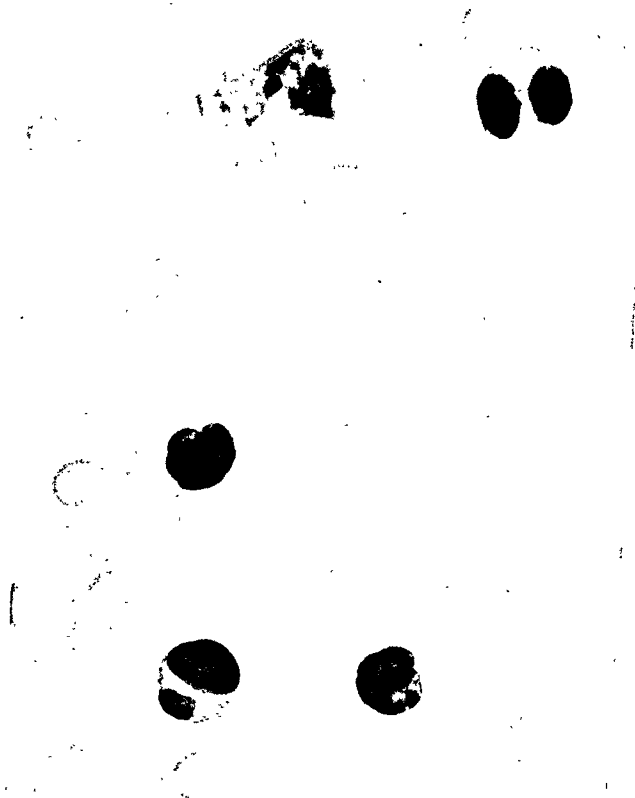


Fig 255 *Lymphocytic Leukemia Chronic Blood* The patient was a middle-aged female with pronounced microcytic anemia and a leukocyte count of 41,100 per cu mm, of which 94 per cent were adult lymphocytes The tip of the spleen reached umbilical level, but superficial lymph nodes were not enlarged While most of the circulating lymphocytes appeared quite normal, those in the field illustrated disclose the nuclear lobation of these cells, usually masked by overlapping of the lobes A disintegrated cell (degenerated lymphocyte "smudge cell," "basket cell") is seen near the top (× 2100)

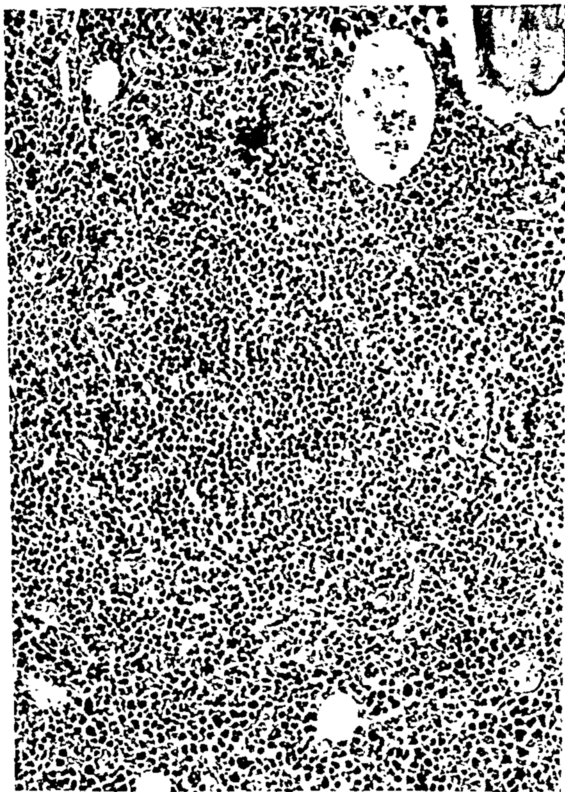


Fig 256 *Lymphocytic Leukemia, Chronic Bone Marrow Section Late in the Disease* The marrow spaces are solidly cellular and occupied in considerable measure by small lymphocytes compactly arranged in more or less circumscribed nodules. The bulk of the field here is taken up by such a lymphoid mass, but the lower right-hand corner shows hyperplastic hematopoietic tissue (× 375)

Fig 257. *Lymphocytic Leukemia, Chronic. Bone Section (Rib) Showing Osteolysis and Tumefaction* The marrow spaces are solidly filled with neoplastic tissue and many trabeculae have been lost. The cortex over the upper aspect has been disrupted, and an extraosseous tumor formed

Fig 258 *Lymphocytic Leukemia, Chronic Bone Marrow Section During Roentgen Therapy* The lymphocytes had previously been closely packed to occupy much of the marrow space. Most of the cells have disintegrated, and small fat vacuoles are beginning to appear in the empty spaces This is usually followed by the reappearance of normal hematopoietic elements which may persist until overgrown again by leukemic cells (× 1000)



Fig 250 ... ably em... dmittec... narrow... ymphoc...



Fig. 260 "*Leukosarcoma*." This situation, like lymphocytic leukemia, lymphosarcoma, Hodgkin's disease, etc., does not represent a neoplastic entity, but rather a variant of the lymphoma complex. The blood (upper panel) of this patient presented a fairly large complement of lymphoblasts, while the bone marrow (middle panel) showed nonspecific hyperplasia. The lower panel pictures a biopsy of a large retroperitoneal tumor classified as a malignant lymphoma of lymphosarcomatous type (upper × 2000, middle × 2280, lower × 750).

Fig 261 *Monocytic Leukemia, Subleukemic Blood* A nineteen-year-old male was admitted to the hospital because of [illegible]

Fig 262 *Monocytic Leukemia, Subleukemic Bone Marrow Smear* (same case as Fig 261) About 80 per cent of aspirated cells were abnormal monocytes similar to those found in the blood A few showed greater immaturity (left) (× 2280)

proteins, sometimes with inversion of the albumin-globulin ratio, although hyperproteinemia has been reported. Total lipids of the blood are considerably increased, but cholesterol is usually normal or decreased. Blood phosphorus is high in chronic leukemia, and increases with treatment, and reverts to normal during remission. Serum phosphatase is also increased over the normal.

Treatment. The treatment of leukemia may be divided into two categories; (1) measures designed to improve the general physical condition of the patient and protect him from infection, and (2) measures designed to destroy the leukemic cells or inhibit their proliferation.

In the first category, *transfusion of whole blood*, the fresher the better, is the most important, and every effort should be made to restore red blood cells and hemoglobin to as nearly normal levels as possible When hemorrhage is due to thrombocytopenia, it is important to use freshly drawn blood for the transfusions The *antiheparin* substances (toluidin blue, protamine) are said to help occasionally in controlling hemorrhage. In acute leukemia, especially the subleukemic and aleukemic forms, the free use of *antibiotic* and *chemotherapeutic agents* is strongly recommended to avoid the ulcerative and gangrenous lesions of the mouth and throat to which these patients are subject. A *high caloric intake* should be prescribed, along with sufficient sedation to keep the patient comfortable.

Radiant energy in the form of roentgen rays or radioactive isotopes is the treatment of choice in patients with chronic leukemia, even the subleukemic forms, with respect to the destruction and inhibition of leukemic cells. The small dose roentgen ray technic is recommended, and it is best to adapt the method to each patient rather than use an empirical plan of treatment for all. We prefer ports over the flat bones, especially in chronic granulocytic leukemia with considerable anemia, as the marrow can usually be cleared of leukemic cells sufficiently that regeneration of the erythrocytic series can come about (Fig. 242). The same applies to chronic lymphocytic leukemia

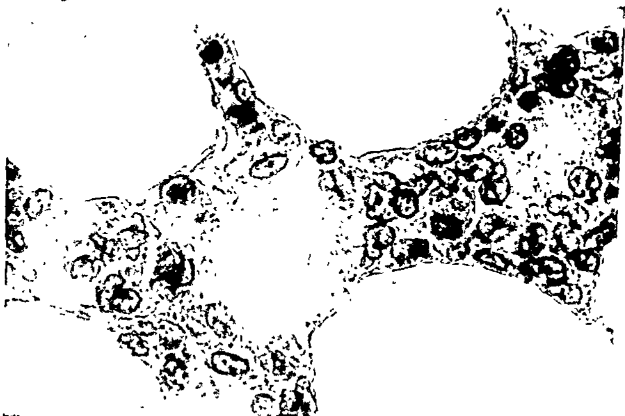


Fig. 263 *Monocytic Leukemia, Subleukemic Tibial Bone Marrow Section* (same case as Figs 261 and 262) The tibial marrow, normally devoid of hematopoietic tissue in the adult, affords a nice demonstration of early leukemic proliferation in the interstices, apparently stemming from preexisting reticulum cells (× 1000)

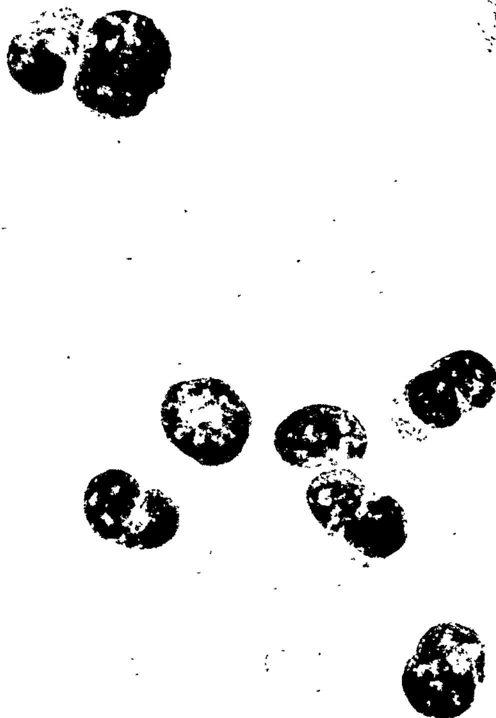


Fig 264 *Monocytic Leukemia, Partially Differentiated Blood* The illness was typical of subacute leukemia of any type Most of the circulating leukocytes were relatively anaplastic, but a few showed nuclear configurations and cytoplasmic pseudopods of monocyte type (× 2280)

proteins, sometimes with inversion of the albumin-globulin ratio, although hyperproteinemia has been reported. Total lipids of the blood are considerably increased, but cholesterol is usually normal or decreased. Blood phosphorus is high in chronic leukemia, and increases with treatment, and reverts to normal during remission. Serum phosphatase is also increased over the normal.

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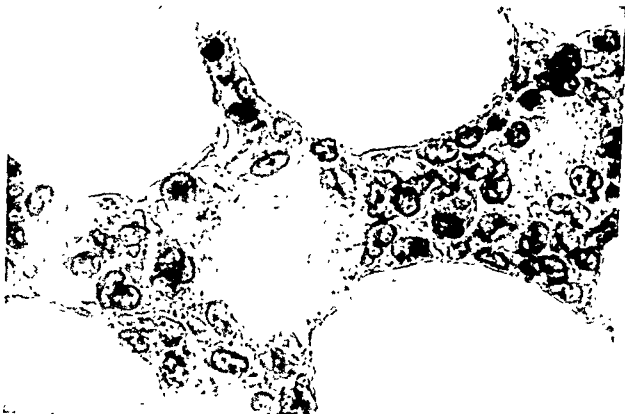


Fig. 263. *Monocytic Leukemia, Subleukemic. Tibial Bone Marrow Section* (same case as Figs 261 and 262) The tibial marrow, normally devoid of hematopoietic tissue in the adult, affords a nice demonstration of early leukemic proliferation in the interstices, apparently stemming from preexisting reticulum cells (× 1000).

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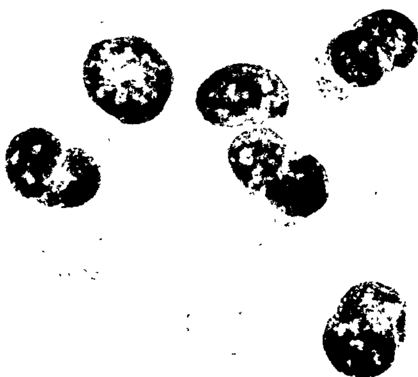


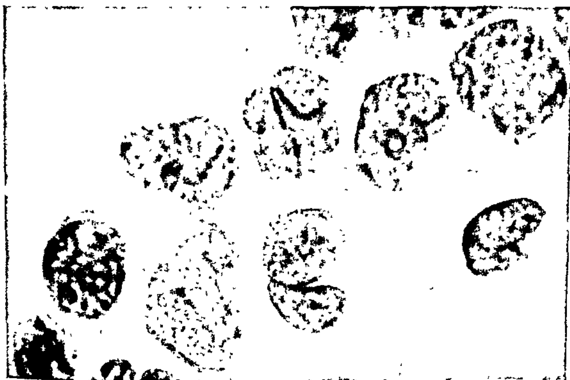
Fig 264 *Monocytic Leukemia, Partially Differentiated Blood* The illness was typical of subacute leukemia of any type Most of the circulating leukocytes were relatively anaplastic, but a few showed nuclear configurations and cytoplasmic pseudopods of monocyte type (× 2280)



Fig 265 *Monocytic Leukemia, Well-Differentiated Type Blood* This fifteen-year-old female developed Bell's palsy of central type two months before admission to the hospital Generally distributed purplish nodular skin lesions appeared over the next few weeks, followed by periorbital swelling and dimmed vision She had fever, night sweats, and substernal pain for a week prior to admission Examination also disclosed patchy induration of oral mucosa, and enlargement of superficial lymph nodes The leukocyte count ranged from 59,000 to 154,400 per cu mm, with 89 to 91 per cent of monocytes, most of which were partially to well differentiated forms She died eight days later The picture shows monocytes in progressive stages of differentiation, proceeding counter-clockwise (× 2280)

when the marrow has been overgrown with lymphocytes (Fig. 258). Secondary attention *is then given an enlarged spleen or lymph node* masses unless by reason of their size they produce symptoms that require earlier relief. One must guard against overdosage of radiant energy, as marrow aplasia may develop (Fig. 243). We have found no better results with the use of radioactive phosphorus (P32), except that radiation sickness has been avoided.

Irradiation is contraindicated in acute leukemia I have seen the tissues swept completely free of leukemic cells by this means, but the bone marrow remained aplastic and the patients died within a relatively short time (Figs 226 and 227).



(× 2280)

Chemical agents of value in treating patients with chronic leukemia are *arsenic* (Fowler's solution), *ethyl carbamate* (urethane), and *nitrogen mustards*. We always employ Fowler's solution primarily and sometimes have been able to control chronic granulocytic leukemia for long periods before it is necessary to begin roentgen therapy

The *folic acid antagonists* (aminopterin, amethopterin, aminoanfol) offer some hope in inducing remissions in the acute leukemias. Aminopterin has been particularly useful in children, and I have seen restoration of an essentially normal bone marrow during the remission. The patients are prone to develop ulcerative lesions of the mouth and throat, sometimes extensive gangrene, and the drug must be administered with caution. These effects are still more marked in adults. Amethopterin, and especially aminoanfol, are less toxic and can be given more freely to adults. I have seen remissions apparently induced by the administration of large doses of brewer's yeast, for no good reason.

Prognosis It is impossible to predict longevity in a patient. Those with the acute form may die in a few days or weeks, others will survive for months and may occasionally have prolonged remissions. The average duration of life in patients with chronic leukemia is between three and four years, but some live from ten to twenty-five years.

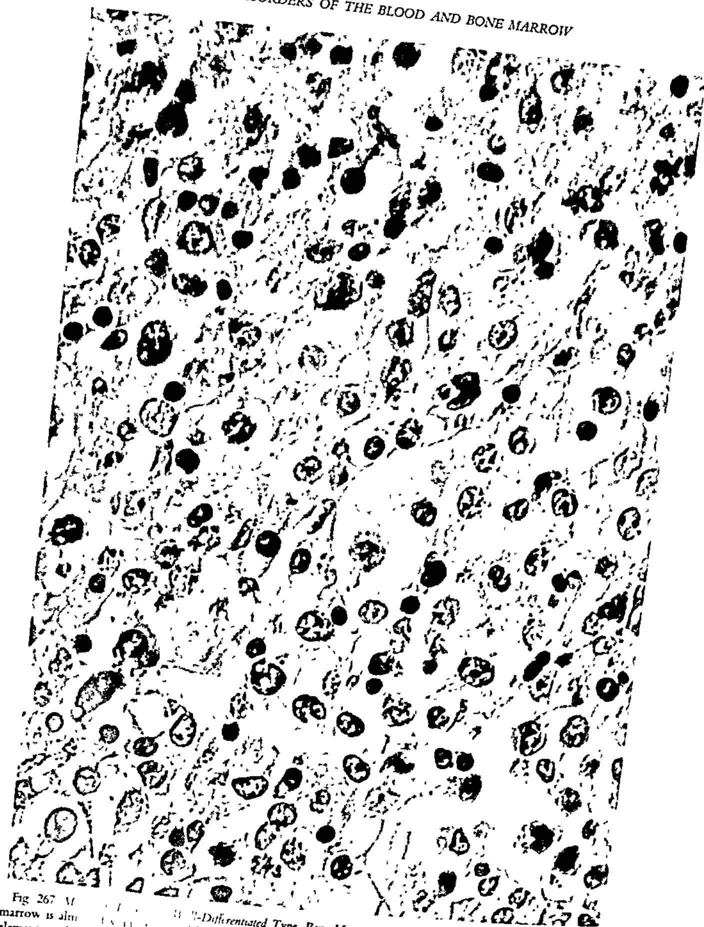


Fig 267 M[illegible] -Differentiated Type *Bone Marrow Section* (same case as Figs 265 and 266) The marrow is alm[illegible] hematopoietic cells The leukemic cells are closely akin to reticulo-endothelial elements, and sh[illegible] the reticular fibrillae of the marrow (× 1000).

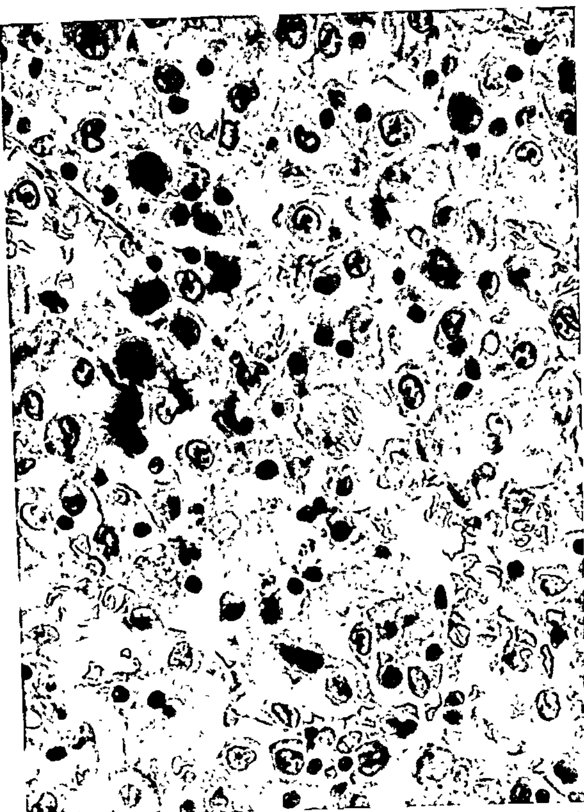


Fig 268 "*Reticulo-endotheliosis*" *Bone Marrow Section* (*Autopsy*) (same case as Fig 269). Death occurred three weeks after the biopsy, despite many blood transfusions in the interim The marrow of all bones examined displayed the changes pictured here, hematopoietic tissue being replaced by cells of varying size, shape, and nuclear configuration, many showed evidence of active phagocytosis of red blood cells and tissue debris A similar proliferation of reticulo-endothelium occurred in the other tissues, notably spleen, lymph nodes, and liver (× 1000)

Fig 269. "*Reticulo-endotheliosis*" *Bone Marrow Smear* The patient developed anemia, leukopenia, and thrombocytopenia which progressed rapidly. The clinical diagnosis was aplastic anemia Aspiration of the sternal bone marrow brought forth masses of large cells with bizarre multiform nuclei and fragile, blue-gray, dusty cytoplasm with ill-defined borders Some cells appeared to form a syncytium (center) (× 1500).



Fig 270 *Plasmacytic Leukemia Blood* The clinical picture in this case did not serve to distinguish it from any other form of acute leukemia Leukocytes ranged from 15,000 to 30,000 per cu mm, and the majority were of the type illustrated The nuclei contain coarse chromatin blocks, and the cytoplasm shows the heavy basophilic material typical of plasmacytes Nuclei were eccentrically placed, and some cells had a central acidophilic zone in the cytoplasms (× 1800)

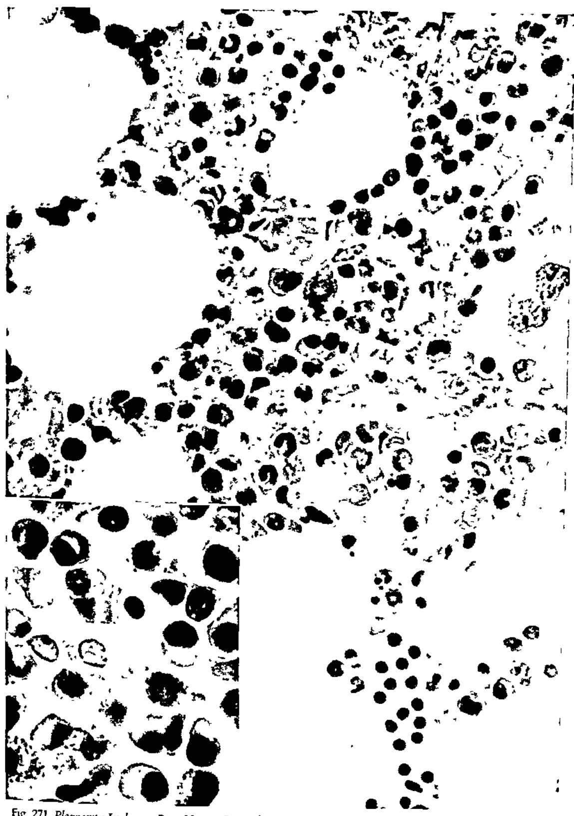


Fig 271. *Plasm...* disease shows a They are readily cluster (× 1000, insert × 2000)

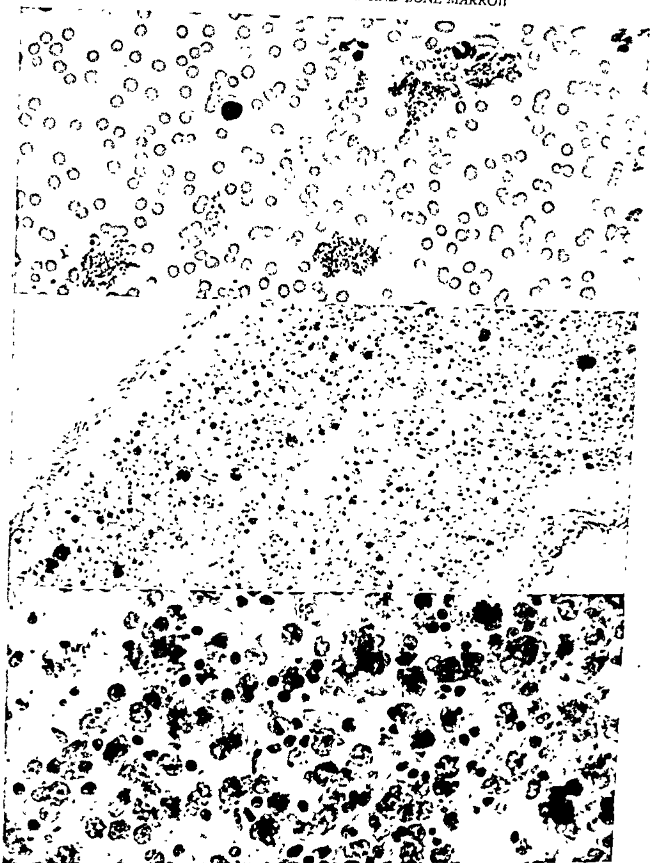


Fig 272 *Thrombocytic Leukemia* The clinical records of this case are not accessible *Upper Panel* The blood smear shows large masses of thrombocytes richly distributed over the slide, no megakaryocytes present in the single smear available for study *Middle Panel* Section through a distended venous channel shows many megakaryocytes in the circulating blood, this may have been a terminal phenomenon *Lower Panel* The bone marrow contains an exceedingly large complement of megakaryocytes, many of them bizarre forms, and megakaryoblasts, there is a paucity of granulocytes, most of the other cells being erythrocyte progenitors (exact magnifications not recorded)

Dr Jacob Furth, Veterans Administration Hospital, Dallas, Texas)

Fig 274 *Mouse Leukemia, Transmissible, Lymphocytic Blood* The leukemic cells are comparable to well-differentiated lymphocytes of man (× 2100) (Slide by courtesy of Dr Jacob Furth, Veterans Administration Hospital, Dallas, Texas)

Fig. 275. *Mouse Leukemia, Transmissible, Monocytic. Blood.* The malignant monocytes differ from normal ones chiefly by the large size of their nuclei and basophilia of their cytoplasm; they are actively phagocytic. They do not invade the peripheral blood in the profusion common to the granulocytic or lymphocytic types, the field shown being unusual in this respect (× 2280) (Slide by courtesy of Dr. Jacob Furth, Veterans Administration Hospital, Dallas, Texas.)

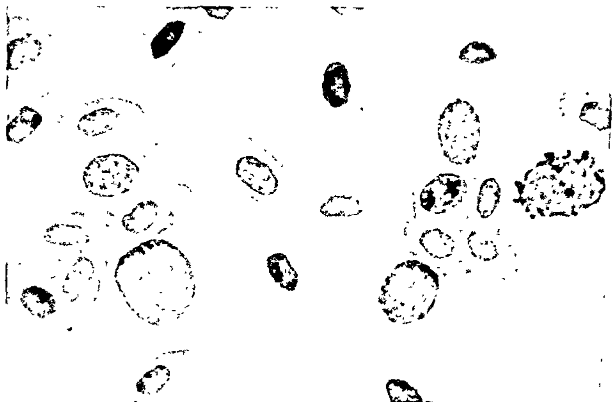


Fig. 276. *Erythroleukosis, Fowl. Blood.* The large round cell in the lower left is an early erythroblast with basophilic cytoplasm. All steps in development between this and adult erythrocytes (oval cells with compact nuclei and acidophilic cytoplasm) may be seen in this single field. An erythroblast in mitosis lies at the right (× 2280) (Slide by courtesy of Dr. Berley Winton, U. S. Department of Agriculture, Regional Poultry Laboratory, East Lansing, Michigan.)

XVI

POLYCYTHEMIA

(Erythrocytosis and Erythremia)

A significant increase in the red blood cell count over normal levels presents a problem that can be solved only through close collaboration of the clinician and the clinical pathologist. Primarily it must be decided if they are dealing with *erythrocytosis* due to compensatory overproduction of red blood corpuscles in response to chronic anoxia or to concentration of blood cells through loss of blood plasma. If these conditions can be eliminated, *erythremia* (polycythemia rubra vera) must be considered. The situation is quite analogous to leukocytosis and leukemia.

ERYTHROCYTOSIS

Relative Erythrocytosis Dehydration resulting from restricted intake or excessive loss of fluids may produce relative erythrocytosis. A shift of plasma into the tissues, such as one finds in shock or following reduction of electrolytes (adrenal cortical insufficiency), sometimes causes marked hemoconcentration, especially in capillary blood. Although the hematocrit level is increased, total blood volume is reduced Leukocyte and thrombocyte counts are elevated in proportion to the erythrocyte count, but immature cells of the several series are absent in the peripheral blood unless provoked by the primary disease. Likewise, the marrow does not show reactive hyperplasia.

Actual Erythrocytosis This is a purely compensatory mechanism to make up for oxygen want in the tissues The major causes are: (1) unsaturation of arterial blood with oxygen (chronic pulmonary disease with defective ventilation, or residence at high altitudes), (2) abnormal pulmonary circulation (congenital heart disease, Ayerza's syndrome), (3) reduced cardiac output (acquired heart disease), and (4) reduced oxygen-carrying capacity of hemoglobin (abnormal combinations with certain chemical agents, see Table 17).

There is a direct relationship between the severity of the causal factor and the erythrocyte count, levels exceeding 10,000,000 per cubic millimeter having occasionally been observed in patients with congenital heart disease where the pulmonary circuit has been bypassed to a considerable degree Reticulocytes may be slightly increased, but nucleated red blood cells are virtually never found in the circulation, a point of some value in differentiation from erythremia Erythrocytes are generally normal in size, and their hemoglobin content is frequently reduced In some patients with a low color index, it has been possible to lessen the red blood cell count by administering iron and restoring hemoglobin to normal. The volume of packed cells is increased, while total blood volume may be normal or elevated depending on the degree to which plasma volume is readjusted. Leukocytosis and thrombocytosis are not features of this condition, whereas they are frequently prominent in erythremia. The bone marrow is hyperplastic, activity being confined largely to cells of the erythrocyte series

ERYTHREMIA

(Polycythemia Rubra Vera)

Erythremia is a chronic progressive disease resulting from purposeless overactivity of the bone marrow and characterized by an absolute

increase in the red blood cell count and total blood volume, generally with an associated leukocytosis. The cause is unknown, but the best evidence points to the disease being a neoplastic one much the same as leukemia. Its relation to leukemia will be mentioned later. Erythremia usually appears between the ages of thirty-five and fifty, with males being the more frequently affected. The incidence is both sporadic and familial, and Jews make up nearly half of the cases. The condition is not so rare as was once supposed, being encountered with increasing frequency because of better diagnosis.

Clinical Manifestations. Most symptoms of erythremia are linked with the sluggish circulation of unusually viscous blood and with the paradoxical tendency toward both thrombosis and hemorrhage. As these processes may affect virtually any combination of tissues to a variable degree in a person, it is obviously beyond the scope of this book to present any but the more common clinical features.

Erythrocyte and hemoglobin levels may be significantly elevated for some time before symptoms appear. The onset is usually insidious and marked by vague complaints of headache, vertigo, nervousness, paresthesias, or merely tiredness; I recall two patients who had been regarded as purely psychoneurotic for several years before a blood count was performed and the disease recognized Perhaps the most characteristic sign, when present, is the dusky red discoloration of the skin, most prominent on the tip of the nose, the cheeks, ears, and extremities, but almost never appearing over the torso. Temperature usually affects the color, which is redder in warm weather and bluer in cold. Not all patients display such discoloration, however Purpuric spots frequently appear in the skin, and acne rosacea is commonly seen

Circulatory stasis or thrombosis in the lower extremities may give rise to erythromelalgia, intermittent claudication, or even gangrene Minor thromboses in the gastric or duodenal wall are said to be responsible for the relatively high incidence of peptic ulcer, while mesenteric thrombosis has been the immediate cause of death in some instances Symptoms referable to the central nervous system may range from the mild ones already mentioned, frequently associated with visual and auditory disturbances, to major encephalomalacia or cerebral hemorrhage. Neurologic signs have sometimes been the same as those of brain tumors. An interesting sidelight to this is the report of several cases of subtentorial tumors associated with polycythemia in which the red blood cell count fell to normal after removal of the tumor.

Bleeding as a result of expansion of the vascular bed is not uncommon, especially from the nose, and along the gastro-intestinal and genito-urinary tracts. The opinion has been expressed that there are alternate periods during which a tendency toward either bleeding or thrombosis may prevail.

The causal relationship of polycythemia to hypertension has been disputed. Certainly, many patients with erythremia have shown neither hypertension nor enlargement of the heart. Most observers believe that the hypertension is fortuitous and dependent on the age of the patient and degree of arteriosclerosis present I am influenced toward an inbetween view by my study of two women with erythremia, both hypertensive and neither with cardiac hypertrophy; their systolic blood pressure curve faithfully followed the red blood cell counts, and their diastolic pressure was never elevated in proportion to the systolic. Apparently any situation may exist in this respect, and there seems to be no need for such categories as Gaisböck's syndrome (polycythemia and hypertension without splenomegaly) Gout has been reported in 5 per cent of cases.

Demonstrable enlargement of the spleen is found in about three-quarters of the cases, and the tip of the spleen occasionally reaches the umbilicus or beyond. The splenomegaly is congestive in nature, infarction with consequent discomfort sometimes occurs. Patients with associated leukemoid reactions tend to have the larger spleens. Engorgement of the liver usually causes enlargement of that organ to a variable degree. Reduction of polycythemia is generally followed by diminution in size of both spleen and liver, although splenic

or portal vein thrombosis is apt to result in permanent enlargement; liver function tests reveal significant parenchymal damage in some instances. The designation of Mosse syndrome for cases of erythremia with cirrhosis of the liver is artificial.

Laboratory Findings. Significant data obtained by laboratory studies are summarized as follows:

TABLE 23

LABORATORY FINDINGS (ERYTHREMIA)

	Erythremia	*Normal*
Hemoglobin	17-24 gm./100 ml.	14-16
Erythrocytes	6-10.5 mil./cu.mm.	4.5-6
Hematocrit	50-80 per cent	40-46
Total blood volume	75-140 ml./kg.	65
Total plasma volume	45-60 ml./kg	
Red blood cell mass	35-100 ml /kg	25-30
Specific gravity	1 075-1 080	1 055-1 065
Viscosity	6-9 (Hess technic)	5
Volume index	1 or less	1
Color index	1 or less	1
Reticulocytes	1-3 per cent*	0.5-1.5
Erythrocyte fragility	Variable	0 46-0.32
Sedimentation rate	Delayed	Varies with method
Serum bilirubin	0.5-5 0 mg /100 ml.	0.5-0 8
Urobilinogen, urinary	0.5-20 mg./24 hr	0.5-3.5
Thrombocytes	0.3-6 0 mil./cu mm.	0.3-0 6
Bleeding time	1-3 min	1-3
Coagulation time, venous	6-15 min.	6-15
Clot retraction	Normal or imperfect	
Leukocytes	6-70 th /cu mm	6-9
Differential count†	Granulocytosis with immaturity	
Gastric analysis	No significance	
Basal metabolic rate	Normal or increased	Minus 10 — Plus 10
Blood uric acid	3.5 mg /100 ml. or more	3 5-4 5

* Actual increase greater than indicated by percentage.
† Nucleated red blood cells frequently encountered.

Analysis of Table 23, beyond the elevated erythrocyte and hemoglobin values, serves to emphasize the diagnostic importance of the red blood cell mass, specific gravity, viscosity, and leukocytosis, with evidence of immaturity in both erythrocytes and leukocytes in the peripheral blood

Bone Marrow. With the exception of bones of the extremities, virtually all fat of the marrow has been replaced by hematopoietic tissue; marrow of the long bones is apt to be only slightly to moderately hyperplastic. Each of the developmental series participates in the hyperplasia, with erythrocyte progenitors generally in the foreground There is no disturbance in the maturation sequence in the red blood cell series, so that intermediate and late erythroblasts and normoblasts predominate, although proerythroblasts and early erythroblasts are found in considerable numbers in patients with exceedingly high red blood cell counts (Fig. 277). Reticulocyte counts on aspirated marrow are generally elevated beyond 2 per cent. Precursors of the granulocytes are also present in abundance, especially in association with a leukemoid blood picture, but the erythrogranulocytic ratio is seldom less than 1.2 and is frequently reversed. Occasionally the granulocyte component is markedly increased, however. As one finds in chronic granulocytic leukemia, the percentage of segmented neutrophils is significantly increased over the normal. Megakaryocytes may be richly distributed throughout the tissue; there is a rough correlation between their numbers and the thrombocyte count in the peripheral blood, although megakaryocytosis can exist without thrombocythemia.

Relation to Leukemia. The interrelationship

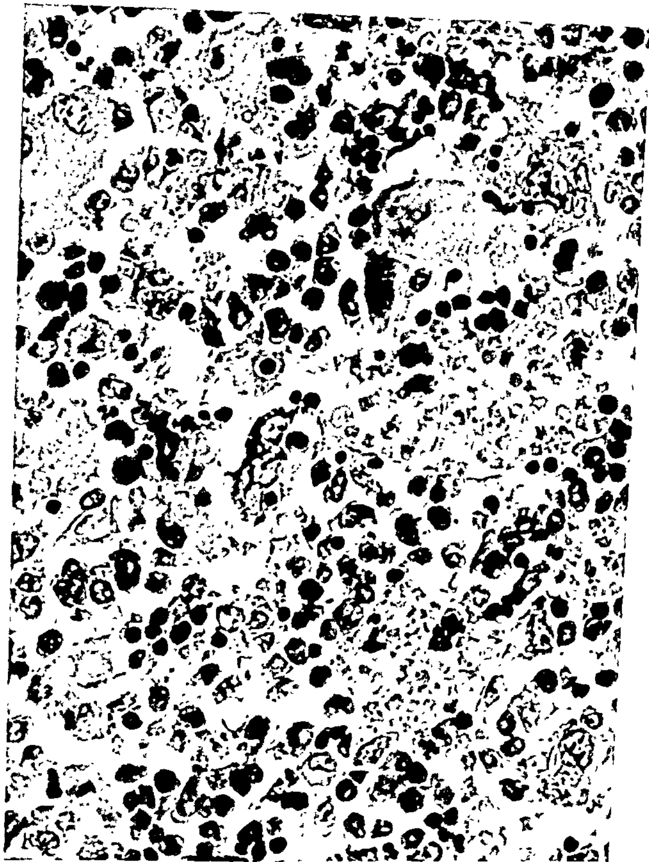


Fig 277 *Erythremia* (Polycythemia Rubra Vera) *Bone Marrow Section* A white female [illegible]

[illegible] hematocrit 80 per cent, WBC 80,700 with immaturity to progranulocytes, Whole blood volume 135 cc kg, Viscosity 1 10 The marrow is diffusely hyperplastic, with all developmental series participating, megakaryocytes are especially prominent (1000) (Slide by courtesy of Dr Archie Baggenstoss, Mayo Clinic, Rochester, Minnesota)

between erythremia and granulocytic leukemia is suggested by the following observations:

1. The development of leukemoid blood pictures in cases of otherwise typical erythremia, with evidences of immaturity among the granulocytes.

2. The development of anemia in some cases of erythremia with a leukemoid blood picture, with persistence of the hyperleukocytosis.

3 The occasional finding at autopsy of extramedullary granulopoiesis resembling the typical pattern of chronic granulocytic leukemia.

4. The report of a few cases in which acute granulocytic leukemia developed as a terminal phenomenon of erythremia.

5 The occurrence of polycythemic phases in patients suffering from otherwise typical chronic granulocytic leukemia.

*I have studied two patients at intervals, in*cluding several bone marrow biopsies in each, and am still not sure whether they should be regarded as having erythremia with a leukemoid reaction or chronic granulocytic leukemia with polycythemic phases

Prognosis Erythremia generally runs a protracted course of twenty years or more, and cases of long duration often display terminal anemia Life is apt to be cut short at some unpredictable time, however, by thrombotic or hemorrhagic episodes. Many patients die of causes quite unrelated to their primary disease, owing to erythremia having developed relatively late in life

Treatment. Radioactive phosphorus (P32) administered intravenously is the treatment of choice An initial dose of 3 to 5 millicuries is injected and the effect observed for about three months, if erythrocyte levels have not reached normal, an additional 1 to 3 millicuries may be given, provided the leukocyte count is not low. In the early days of isotope therapy, I gave a patient 7 millicuries of P32 by mouth, followed by 5 millicuries intravenously three days later. The ultimate effect was a six year remission, but the effect on my nerves over the first few months was serious when the red blood cell and leukocyte counts dipped so low that I considered the advisability of blood transfusion Her subsequent relapse was minor and has been controlled by roentgen therapy.

"Spray irradiation" covering a field from the neck to the knees accomplishes much the same effect as isotope therapy, although it is more time-consuming and often induces radiation sickness The daily dose is 20 to 30 air r (0 5 mm Cu and 1 mm Al filtration) making up a total of 300 to 500 r for any one series, divided between the anterior and posterior aspects of the body

*Acute symptoms of the disease may be alle*viated temporarily by venesection, but no more than 500 ml. of blood should be removed at any one time, nor should this be repeated oftener than once a week; the erythrocyte count and hematocrit should never be reduced below normal by this means. Normal blood levels can also be attained by administration of acetyl phenylhydrazine in oral doses of 0 1 gm daily for ten days, followed by a rest period of ten days and subsequent dosage regulated by the response in the individual patient Fowler's solution (potassium arsenite) is often effective in controlling red blood cell proliferation, beginning with 1 drop three times daily and increasing one drop per dose per day to a maximum of 15, then attempting to fix a maintainance dose of 5 to 10 drops for the given individual Gastro-intestinal symptoms frequently arise as a contraindication to continued use of arsenic, however,

XVII

TECHNIC

There are so many sources of information on technical methods for examination of the blood, with most procedures so well standardized, that their inclusion in this book would be superfluous I shall only repeat the truism that the results of tests are only as valuable as the care that goes into their performance.

The clinical pathologist should observe and coach his technologists until he gains full confidence in their manner of work, and they must be instructed to call his personal attention to any unusual finding. Thus, technologists should serve as a screening medium and not be delegated to conduct the division of hematology, as so often happens; a practice that is not fair to them or to the patients. On the other hand, everyone benefits when the clinical pathologist takes the trouble to discuss with his technologists diagnostic or therapeutic problems presented by certain patients, the solution of which may hinge on their efforts. Interest in patients relieves the machine-like routine of the technologists' day and is good for technic.

As some laboratories seem to have difficulty in obtaining good preparations of bone marrow, the methods that we have found satisfactory will be described in detail *It is important that the person responsible for examination of the marrow be the one to obtain the specimen.* This is generally the pathologist, whose activities have long since ceased to be confined to the laboratories and the autopsy room.

BIOPSY OF THE BONE MARROW

Sites. Through habit we nearly always select the *sternum* and enter the marrow cavity at the level of the second or third interspace in the midsternal line. Because the operator is mindful, or should be, of the structures that lie beneath the sternum, *spinous processes of the upper lumbar vertebrae* and the *crest of the ilium* have been recommended as less hazardous sites of biopsy. They have the additional advantage that the patient is less aware of what is going on. The marrow samples from all three bones are equally representative, so that the choice is a matter of personal preference. *Ribs* have also been aspirated, but there is some risk of entering the pleural cavity. In children up to the age of five years, the tibia is the bone of choice, entry into the upper third of the diaphysis being made from the medial aspect. The crest of the ilium is a better site in older children (Fig. 279).

Aspiration versus Trephine. In the earlier days of marrow biopsy, I preferred to remove a button of ventral cortex from the sternum with a trephine and prepare sections from the marrow surface (Fig. 278). Additional fragments of marrow could be scooped out for study by a variety of methods (supravital staining, tissue culture, and so on), and for preparation of streaks and imprints uncontaminated with peripheral blood. This is without doubt the procedure of choice from the scientific viewpoint, affording a relatively large quantity of pure marrow with which to work. It must be performed in the operating room, however, adding expense for the patient and time for the operator. In hemorrhagic states, bleeding may be hard to control and a postoperative hematoma may form. Finally, one hesitates to repeat the biopsy and multiply the discomfort and scars, however small We now

employ trephine biopsies only when repeated aspirations have failed to obtain adequate samples of marrow.

To be completely successful, a biopsy should provide material from which both smears and histologic sections of marrow can be prepared. As I gained experience with the aspiration method, I found that both requirements could be fulfilled in most instances. The fluid was generally rich in cells (or fat, if the marrow was hypoplastic) and contained a minimum of peripheral blood; tiny fragments of tissue could usually be isolated, fixed, and sectioned (Figs. 281 and 282). In the best of hands, the aspirate will sometimes be made up largely of peripheral blood and the procedure must be repeated; again, a succession of "dry taps" suggests osteosclerosis or myelofibrosis, and a trephine biopsy is indicated. Aspiration biopsies can be repeated a number of times in the same person, and virtually any patient will admit that he prefers the operation to a trip to the dentist.

Technic of Aspiration Biopsy. Some operators treat the procedure as lightly as they would a venepuncture. We probably err on the opposite side and occasionally provoke an ill-concealed smile at our efforts to maintain rigid surgical asepsis throughout this "medical operation." Nonetheless, we sleep better by having done so, as osteomyelitis in an already sick patient is not pleasant to contemplate.

The patient is given a sedative (morphine sulfate and scopolamine hydrochloride hypodermically in doses appropriate for the size and age of the patient, and the degree of anemia present) one-half hour before operation; the skin is washed and shaved, if necessary. The skin is finally painted with antiseptic solution (Arnold's solution in our institution), which is partially removed with alcohol. The operator scrubs his hands and wrists for ten minutes, rinses them in alcohol, and wears a sterile gown and gloves Sterile towels are laid closely around the selected site of aspiration, and an "eye sheet" is used as an overdrape. The skin, subcutaneous tissue, and periosteum are generously infiltrated with the local anesthetic of choice, using a 25 gauge needle about 3 cm. long While infiltrating the periosteum, one should probe the density of the bone cortex. In some patients with markedly hyperplastic marrow, the cortex will be thin, soft, and readily penetrated by the fine needle; under these circumstances the usual "give" may not be felt when the biopsy needle enters the marrow cavity, and the possibility of pushing the needle through the entire bone can be avoided if this is anticipated. After several minutes the skin is nicked with a straight slender bistoury which is thrust through to the bone at an angle of 45 degrees to the skin surface. The periosteum is incised with the knife tip and the knife withdrawn. From this point the procedure varies according to the type of biopsy needle employed.

When the bone cortex seems to be of the usual density or harder, we use the Turkel instrument consisting of an outer guide needle of 14 gauge with a sharp beveled point and stylet, whose stylet can be replaced by an inner trephine needle of 17 gauge, also with its own stylet. The guide needle with stylet in place is passed through the incision tract and the point firmly fixed in the cortical bone, using pressure and a slight to and fro rotary twist. The stylet is withdrawn and the trephine needle (without its stylet) is inserted and twisted through the bone cortex with a clockwise rotary motion until its hub approximates that of the outer needle; it is held in this position while the outer needle is rotated and pushed slightly deeper into the bone cortex to assure its firm fixation. The inner needle is then withdrawn and the plug of bone dislodged from the lumen with the small stylet (the plug can be saved for sectioning, although there is generally very little marrow attached) The inner needle is again passed into the marrow cavity with a twisting movement and a 10 ml syringe attached. The plunger is quickly withdrawn to about the 6 ml mark and the suction released as soon as bloody fluid appears at the butt of the syringe, allowing the plunger to slip back so that not [illegible] The

the contents of the needle sucked into the syringe before it is detached The syringe is then passed to an assistant who prepares smears on scrupulously clean micro slides as one would a

blood smear. Limarzi* suggested a nice method whereby a small drop of marrow fluid is sandwiched between two slides held at right angles to one another, so that a marrow film occupies one end of a slide and a blood film similarly prepared the other, with the central space for labeling.

Some people prefer to withdraw 1 ml of marrow and admixed blood into a syringe whose barrel has previously been moistened with sterile heparin solution with a slight excess at the butt. The blood-marrow mixture is expelled into a Wintrobe tube and centrifuged for five minutes at 2000 rpm The top fat and most of the plasma are removed and discarded, and the buffy cellular layer carefully drawn off through a pipet for the preparation of smears. In either case the smears are rapidly air dried by waving the slides and *immediately fixed for several minutes in absolute* methyl alchohl in a Coplin jar. Stored in a dustproof box, they can be stained at one's leisure.

We prefer a combination of May-Grunwald and Giemsa stains (Wright's stain can be substituted for the May-Grunwald) according to the following procedure:

May-Grunwald stain	3 min
Add an equal amount of triple distilled water	1 min
Drain, do not rinse	
Overlay with dilute Giemsa stain (1 ml stock stain in 30 ml triple distilled water)	12 min
Rinse with a few dips in triple distilled water and check intensity of stain with the microscope, if too blue, rinse a little more, if too pallid, repeat the procedure using half times	
Tilt slides and air-dry	
Apply coverslip, using a neutral mounting medium.	

By this technic, bone marrow and blood films are equally well stained.

Limarzi's directions for staining the combined blood and marrow films are as follows "The marrow film is covered with Wright's stain, which is diluted with double or triple distilled water after three minutes After about fifteen minutes, the blood film is covered with Wright's stain, which is diluted after ninety seconds. After three minutes, the entire slide is washed until clear. The total staining period should not exceed twenty minutes."

One should employ either of these or any other method that has given consistently good results in the laboratory. Never be satisfied with smears that are too deeply or too feebly stained, much less base a diagnosis on imperfect preparations.

Finally, it is necessary to evaluate the degree to which the marrow has been diluted with peripheral blood I have seen the diagnosis of aplastic anemia erroneously made from a marrow aspirate which was mostly blood. Again, the marrow in infectious mononucleosis has been described as being rich in abnormal lymphocytes, which were actually derived from aspirated blood.

To obtain *marrow for sectioning*, the inner *needle is reinserted through the guide needle* which has been tilted to a slightly different angle, twisting the inner needle as before when the marrow cavity is being entered. Attach a fresh heparinized syringe and pull hard on the plunger, withdrawing when no more than 0 2 ml. of bloody fluid has been obtained. Express the aspirate into a small clean watch crystal. Additional aspirates may be secured in the same fashion, with the needle at still different angles, and added to the first. The excess blood is then withdrawn, using a fine capillary pipet, and the watch crystal transferred to the stage of a dissecting microscope, with the light transmitted through the liquid from below (if a dissecting microscope is not available, a hand lens will suffice, still using transmitted light). Fragments of marrow are visible by this means and can be drawn into the tip of a small pipet with attached rubber bulb. At this point one of two technics can be used The fragments may be transferred to a piece of lens paper where they are nested and tied securely with thread; the small paper bag is fixed in Zenker's fluid for four to six hours and carried through the various solutions to the point of embedding in paraffin, then removed with fine forceps and embedded as one would any tissue. The technician must use care not to cut through the fragments while leveling the face of the paraffin block The alternate method suggested by

*J Lab & Clin Med, 32 732, 1947

Berman and Axelrod* provides a specimen which is easier for the average technologist to embed and section. A small amount of powdered topical thrombin is placed on a glass slide, dissolved in a drop of distilled water, and allowed to dry. The bits of marrow are transferred to the area coated with thrombin and gently pushed into close approximation. Three or four drops of heparinized human plasma are added to the marrow, thus forming a firm clot by contact with the thrombin The clot containing the marrow fragments is readily lifted from the slide and placed in Zenker's solution. Standard technic for the preparation of thin paraffin sections is used from this point.

Mertens† advocates aspiration of about 2 ml. of bloody fluid and holding the syringe vertically until clotting occurs The clot is fixed, and sections are made through the top surface, by this means including marrow fragments that have floated before the blood clotted (Fig. 282).

After removal of the paraffin and mercuric chloride from the sections some are stained with hematoxylin and eosin, others with Giemsa's stain. The latter are soaked overnight in a 1:30 dilution of stock Giemsa solution in triple distilled water, differentiated in 95 per cent alcohol, and rapidly dehydrated in three changes of absolute alcohol, cleared in xylol, and mounted in clarite or some other neutral mounting medium. Differentiation must be controlled under the microscope with each slide. Nuclei should be a distinct blue and sharply defined, while the clear red of erythrocytes and eosinophil granules is a good guide on the opposite side.

It is sometimes necessary to adjust the pH of the 95 per cent alcohol in which the sections are differentiated. If the tissue is stained too blue, add a drop of glacial acetic acid to the alcohol; if too red, add 5 per cent sodium carbonate solution. The optimum pH is about 6 8.



Fig 278 *Bone Marrow Biopsy, Trephine Technic Section* The marrow surface of a trephined button from the sternum affords a large area (1 cm diameter) for study (× 10)

* Am J Clin Path, *17* 61, 1947

† Am J M Sc, *210* 630, 1945

When the bone cortex seems to be soft we use the Tocantins needle, which has a sharp beveled point, a stylet, and an adjustable guard A 2.5 cm. length is available for the average panniculus, and a 4 cm. for obese patients. The point is fixed in the cortical bone, employing

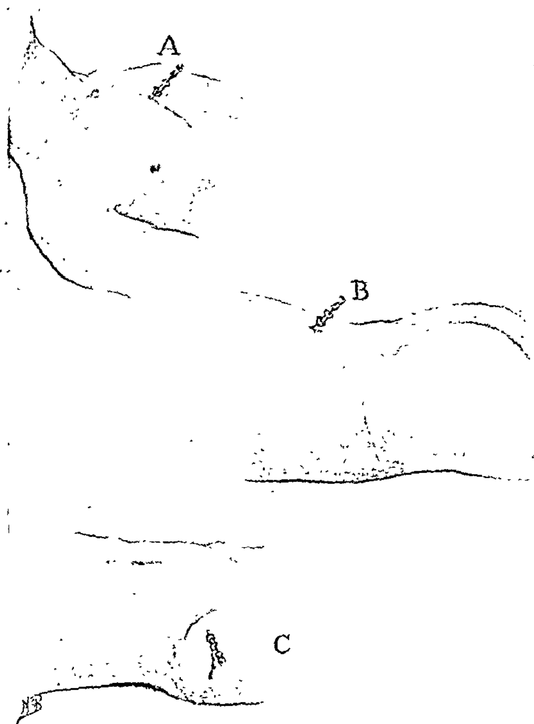




Fig 280 *Site of Sternal Puncture* The exact time elapsed between the marrow aspiration and death is not known, but it probably did not exceed three or four days, as the reparative process is not well established. A considerable quantity of marrow was evidently removed through the needle.

the technic already described, and the guard set about 4 mm. above the skin surface. The needle is gently forced through the sternal cortex, using a slight to and fro motion, being sure that the stylet is firmly in place as the bone is entered; otherwise the stylet will be pushed back and the lumen of the needle clogged with a plug of bone. In most patients, a sudden "give" marks entry of the needle into the myeloid cavity, but one cannot depend on this sign. If distance indicates that the needle should have entered the marrow, withdraw the stylet and apply negative pressure with the syringe. When the marrow sample is obtained, proceed in the manner detailed previously.

The so-called "University of Illinois sternal puncture needle" used by Limarzi has the advantages of a fixed stylet and a Luer-lok adapter, but the type of guard requires vertical insertion into the bone, rather than at an angle, and I am a little hesitant at using it on soft bone.

Technic of Trephine Biopsy Using rigid aseptic precautions in the operating room, the skin and subcutaneous tissues are infiltrated with local anesthetic solution for a 3 cm. radius around the proposed site of biopsy (generally the level of the attachment of the third rib to the sternum). A 2 cm sagittal incision is made down to, but not including the periosteum. The needle is inserted beneath the periosteum, and the subperiosteal space is infiltrated over the operative field. A crucial incision is made through the periosteum, and the bone exposed for an area corresponding to the size of the trephine (5 mm to 1 cm. diameter). The ventral table of the sternum is cut with the trephine, the marrow cavity has been entered when a slight downward thrust is experienced (care must be exerted not to continue drilling through the dorsal table into the mediastinum). The trephine is tilted slightly back and forth in sagittal and transverse planes, thus breaking the fine trabeculae of the underlying cancellous bone. The trephine is then withdrawn, the bone

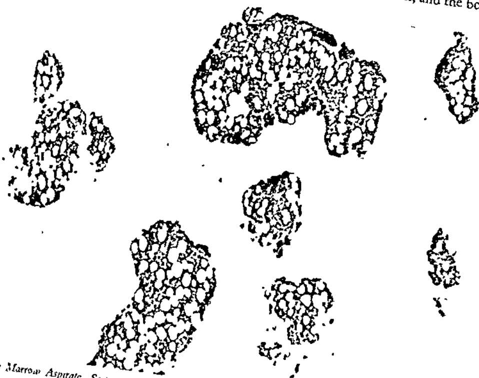


Fig. 281 *Bone Marrow Aspirate. Section of Teased Fragments.* Bits of marrow separated from aspirated blood after fixation can be sectioned and frequently give an excellent picture of the hematopoietic pattern. This patient was suspected of having lymphocytic leukemia, a skin biopsy having been diagnosed as leukemia cutis, but his bone marrow was found to be quite normal (× 50)



Fig 282. *Bone Marrow Aspirate, Mertens' Technic Section* By this flotation technic aspirated fragments of marrow can be sectioned within the blood clot. In this case of acute lymphocytic leukemia the marrow is composed of a uniform mass of lymphocytes (× 250)

Fig 283 *Poor Technic Bone Marrow Smear.* About 10 cc of material (mostly blood) were aspirated from the sternum and placed in a tube containing heparin. After standing for several hours the solution was centrifuged, and smears were made from the buffy coat Cell details are badly blurred, and granules are spilled into the plasma. It would appear that the picture is out of focus, but the several red blood cells in the field are sharply defined (× 2280)

Fig 284 *Poor Technic Bone Marrow Smear* A fragment of marrow was mixed with several drops of blood serum and smears prepared from the mixture Hematopoietic cells were partially broken, and the composite of serum and marrow fat interfered with staining (× 1500)

Fig 285 *Poor Technic, Bone Marrow Smear* The slide was briskly scrubbed with a curetted specimen of bone marrow The results hardly justify the operation (× 300)

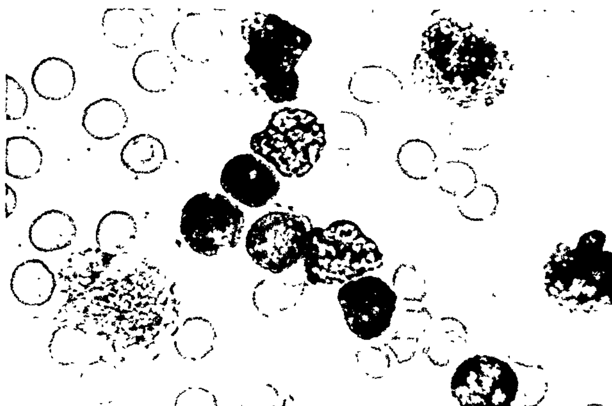


Fig 286 "*Smudge Cells*" ("Basket Cells") *Blood* Blood smear from a case of acute lymphocytic leukemia shows various steps in the formation of so-called "smudge cells," which result from partial breakdown of the more fragile immature leukocytes of any type during preparation of the smear (× 1500)

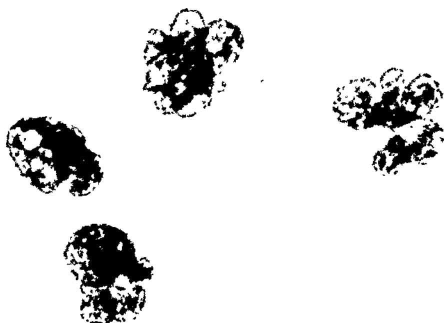


Fig 287 *Oxalate Artefact, Blood Smear* A sample of venous blood was taken from a patient with acute granulocytic

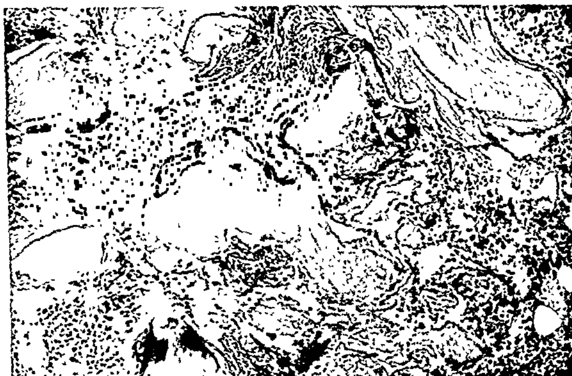


Fig 288 "*Sawdust*" *in Marrow Section* Bone dust is frequently ground into the marrow spaces in specimens removed at autopsy with a coarse handsaw To avoid this, the surface to be sectioned should be trimmed at right angles to the sawed surface after the bone has been decalcified (× 250)

button will either come away with it or can be readily removed with tissue forceps. The margin and floor of the exposed myeloid cavity are scraped with a sharp bone curet. The button and curettings are handed to the assistant who takes charge of the technical details to be mentioned. The bone defect is packed firmly with gauze until all oozing has ceased. The periosteum is drawn back and may (but need not) be held in place by a 00 plain catgut suture through the four ends The fascia and skin are sutured in the usual fashion (in women, a carefully laid subcuticular skin suture may be preferable).

Fragments of marrow are drawn through a serpentine course across clean slides; imprints are made between the loops of the streak. Other bits can be used for supravitally stained preparations or for tissue culture.

The streaks are stained in precisely the same manner as smears of aspirated marrow. The bone button and residual curettings are fixed in Zenker's solution, and decalcified for twelve to twenty-four hours in the following solution:

Formic acid
Sodium citrate (20 per cent aqueous solution) } equal parts

After washing for one hour in running water, sections are prepared and stained according to the method already described.

Poor Technic and Artefacts. A few examples of inadequate handling of marrow specimens are shown in Figs 283, 284, and 285, and several artefacts frequently encountered are seen in Figs. 286, 287, and 288 In addition to the latter, one sometimes finds coarse basophilic globules of precipitated stain superimposed on leukocytes, simulating organisms or inclusion bodies; these can be readily eliminated by decolorizing the slides in absolute methyl alcohol and using a fresh batch of stain. If a patient is to be subjected to critical hematological study, it is imperative that the clinical pathologist makes certain that the best technical methods are employed.

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NOTE Page numbers in *italics* refer to illustrations Words in *italics* refer to therapeutic agents